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Haemophilus Influenzae Strains

Expression of Antibiotic Resistance

Highlights

Cerebrospinal Meningitis Outbreak

Genotypes of Streptococcus Pneumoniae

Discovering Thoughts, Inventing Future

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Pneumococcal Meningitis Outbreak Tracked to PCR Confirmed Genotypes of *Streptococcus Pneumoniae* in Parts of Northern Nigeria

By Iseimokumo Christopher Peletiri, Grace Mebi Ayanbimpe & Eugene Ifeanyichukwu Ikeh

University of Jos

Abstract- Pneumococcal meningitis caused by Streptococcus pneumoniae strains has been reported as the third primary aetiology of bacterial meningitis in parts of Northern Nigeria. However, information on the genotypes of *S. pneumoniae* strains circulating in Northern Nigeria is unavailable in the literature. Genotyping is being practiced widely in medical microbiology and has been shown to be an invaluable tool in tracking strains responsible for disease outbreaks. We aimed at determining the genotypes of *S. pneumoniae* tracked to a pneumococcal meningitis outbreak in parts of Northern Nigeria. The multilocus sequence typing (MLST) scheme, a DNA sequencing based genotyping method of sequencing segments of seven internal housekeeping genes, was used. The PubMLST.org database and Bacterial Isolate Genome Sequence Database software was equally used.

Keywords: pneumococcal meningitis, outbreak, genetic status, genotypes, streptococcus pneumoniae, northern Nigeria.

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Pneumococcal Meningitis Outbreak Tracked to PCR Confirmed Genotypes of *Streptococcus Pneumoniae* in Parts of Northern Nigeria

Iseimokumo Christopher Peletiri [α], Grace Mebi Ayanbimpe [σ] & Eugene Ifeanyichukwu Ikeh [ρ]

Abstract- Pneumococcal meningitis caused by Streptococcus pneumoniae strains has been reported as the third primary aetiology of bacterial meningitis in parts of Northern Nigeria. However, information on the genotypes of *S. pneumoniae* strains circulating in Northern Nigeria is unavailable in the literature. Genotyping is being practiced widely in medical microbiology and has been shown to be an invaluable tool in tracking strains responsible for disease outbreaks. We aimed at determining the genotypes of *S. pneumoniae* tracked to a pneumococcal meningitis outbreak in parts of Northern Nigeria. The multilocus sequence typing (MLST) scheme, a DNA sequencing based genotyping method of sequencing segments of seven internal housekeeping genes, was used. The PubMLST.org database and Bacterial Isolate Genome Sequence Database software was equally used. Of the eight genotyped isolates, three (37.5 %) had detectable genotypes: genotype *aroE* in two cases (25 %), found circulating within serotype 4 (*Wzy* 4); and genotype *gki*, one isolate (12.5 %) found circulating within serotype 5 (*Wzy* 5). The MLST results provided an overview of circulating *S. pneumoniae* genotypes and their genetic status, which is a piece of important information for public health strategies such as vaccination. To develop more effective vaccines, it is imperative that the candidate vaccines must be evaluated against a set of carefully selected genotypes, which are representative of the pathogen isolated.

Keywords: pneumococcal meningitis, outbreak, genetic status, genotypes, streptococcus pneumoniae, northern Nigeria.

I. Introduction

The aetiologic agent of pneumococcal meningitis, Streptococcus pneumoniae has been reported as one of the most common causes of bacterial meningitis beyond the newborn period [CDC, 2011, Chap. 2]. In a very recent study by Peletiri and colleagues, *S. pneumoniae* was reported as the third primary aetiology of bacterial meningitis in parts of northern Nigeria. The authors reported encountering four serotypes of *S. pneumoniae* including serotype 1 (Wzy 1), serotype 4 (*Wzy* 4), serotype 5 (*Wzy* 5), and serotype 9 (*Wzy* 9) as the offending serotypes [Peletiri et al., 2021b]. Meanwhile, some authors had earlier reported serotypes 1, 5, and 19F [Kwambana-Adams et al., 2018] and serotypes 6, 19, and 20 [Suleiman et al., 2018] in Northern Nigeria. Information on the circulating genotypes of *S. pneumoniae* in Northern Nigeria is unavailable in the literature. Genotyping is also known as DNA fingerprinting [Wenjun et al., 2009].

The multilocus sequence typing (MLST) methodology is a DNA sequencing based genotyping method that generates the original sequence of nucleotides and discriminates among bacterial strains directly from polymorphisms in their DNA [Chan et al., 2001]. The MLST scheme indexes the sequence of seven internal housekeeping gene fragments to identify bacterial genotypes and associate them with biological properties [Chan et al., 2001; Jolley et al., 2004; Maiden et al., 1998]. MLST scheme is available for *S. pneumoniae* based on DNA sequencing of fragments of seven housekeeping loci *(aroE, gdh, gki, recP, spi, xpt,* and *ddl)* [Enright & Spratt, 1998]. The assignment of alleles at each locus is carried out using the MLST website (http://www.pubmlst.streptococcuspneumoniae).

The American Society for Microbiology Journals (journals.asm.org/nomenclature) gave explicit instructions to authors on bacteria genetic nomenclature and genotype designations. The genetic properties of bacteria are described in terms of phenotypes and genotypes. The phenotype describes the observable properties of an organism. The genotype refers to the genetic constitution of an organism, with reference to some standard wild type. Genotype designations are indicated by three-letter locus symbols with lowercase italic (e.g., *ara, his, rps)*. If several loci govern related functions, these are distinguished by italicized capital letters following the locus symbol (e.g., *araA, araB, araC*). We aimed at determining the genotypes of *S. pneumoniae* tracked to a pneumococcal meningitis outbreak in parts of Northern Nigeria.

II. Materials and Methods

a) Ethics Approval and Consent to Participate

Ethical approval was obtained from the Health Research Ethics Committees of National Hospital, Abuja, Nigeria (NHA/EC/034/2015); Federal Capital Development Authority Health Services, Abuja, Nigeria

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(FHREC/2017/01/27/03-04- 17); Kebbi State Ministry of Health, Nigeria (MOH/KSREC/VOL.1/56/No101.3/2015); Plateaun State Ministry of Health, Nigeria (MOH/MIS/202/VOL.T/X, 2017); Sokoto State Ministry of Health, Nigeria (SMH/1580/V.IV, 2017), and Zamfara State Ministry of Health, Nigeria (ZSHREC/ 02/03/2017) [Peletiri et al., 2021a; 2021b]. Written informed consent for the storage and future use of the unused sample, and sample material and data transfer agreement were also obtained [Peletiri et al., 2021a; 2021b].

b) Sample Size Determination

The sample size was calculated using the Cochran formula [Cochran, 1977] for calculating simple proportion. At 0.05 alpha level of significance, 95% confidence level and patient population size of seventy-seven and a previous prevalence of 13.7 %, a sample size of 181.7, which was adjusted to 210 samples after calculating 10 % attrition [Peletiri et al., 2021a; 2021b]. The subjects were recruited consecutively until the sample size was attained [Peletiri et al., 2021a; 2021b].

c) Sample Collection

Cerebrospinal fluid samples collection was as previously reported [Peletiri et al. 2021a].

d) Extraction and Quality Check of Metagenomic DNA

Metagenomic DNA extraction methodology and quality check methods were as reported previously [Peletiri et al., 2021a].

e) Multiplex Real-Time PCR for S. pneumoniae Detection

Multiplex Real-time PCR protocol for molecular detection of *S. pneumoniae* was as previously reported [Peletiri et al., 2021b].

f) Singleplex Real-time PCR for S. pneumoniae Characterization

Singleplex Real-time PCR protocol for molecular characterization of *S. pneumoniae* was as previously reported [Peletiri et al., 2021b].

g) Sample Selection

Of the appropriately characterized 12 *S. pneumoniae* serotyped strains with singleplex Real- time PCR as reported by Peletiri et al., (2021b), eight (66.7 %) were properly selected for genotyping to ensure both geographical coverage (spread) and serotype representation or distribution.

h) Multilocus Sequence Typing (MLST) Protocol for S. pneumoniae Genotyping

The Real-time PCR method of Multilocus Sequence Typing (MLST) using SYBR chemistry, is designed to genotype selected genotypic markers of *S. pneumoniae*. The assay detects seven genotypic markers: aroE, gdh, gki, recP, spi, xpt, and ddl [Enright & Spratt, 1998; Maiden, 2000; Maiden et al., 1998], as in Table 1. The primers used for amplification by Real-time PCR were: aroE-F (5'- GCCTTTGAGGCGACAGC-3'), and aroE-R (5'TGCAGTTCAGAAAACATATTTCTAA-3'); gdh-F (5'-ATGGACAAACCAGCNAGCTTT-3'), and gdh-R (5'-GCTTGAGGTCCCATGACTNCC-3'); gki-F (5'-GGCA-TTGGAATGGGATCACC-3'), and gki-R (5'-TCTCCCGC-AGCTGACAC-3'); recP-F (5'-GCCAACTCAGGTCATCC-AGG-3'), and recP-R (5'-TGCAA-CCGTAGCATTGTAAC-3'); spi-F (5'-TTATTCCTCCTGA-TTCTGTC-3'), and spi-R (5'-GTGATTGGCCA-GAAGCGGAA-3'); xpt-F (5'-TTATTA-GAAGAGCG-CATCCT-3'), and xpt-R(5'-AGATCTGCC-TCCTT-AAATAC-3'); ddl-F (5'-TGCCTCAAGTTCCTTATG-TGG-3'), and ddl-R (5'-CACTGGGTGAAAACCATGG-CAT-3').

All primers were synthesized by Eurofins, Germany. Primers were supplied lyophilized. Primers were first reconstituted to 100 µM (working stock) following the manufacturer's instructions and working concentrations of 10 µM prepared using DNA elution buffer (or TE buffer) as diluent.

i) MLST Protocol Set-up

i. *Sample Requirement*

Metagenomic DNA (mDNA) samples of serotyped *S. pneumoniae* with singleplex Real-time PCR, stored at – 20 OC (or at – 80 OC) until required for testing.

j) Reagents and Materials

10 µM *S. pneumoniae* primer mixes labelled *aroE, gdh, gki, recP, spi, xpt,* and *ddl* mix, respectively. qPCR Master Mix (SYBR); PCR water (Nuclease free water); ABI One Step Plus Real-time PCR System (Thermofisher, UK); ABI 96 well qPCR plate; P10, P100, and P1000 pipettes and tips; Thermal seal for PCR microtitre plate; Cold rack; Refrigerated centrifuge with plate holder (Heraeus, UK).

k) Setting up Reaction

A worksheet was created according to the number of samples to be tested. The ABI 96 well plate was placed into a plate holder on a cold rack. Note: Each genotypic marker was tested at a time. So, for each sample, there were seven separate reactions. Into each well, 15 µL of qPCR Master mix / Primer was dispensed. Five (5) µL of sample (mDNA), Positive control and Negative control (PCR water) was added into the appropriate well. The plate was sealed with a thermal seal and centrifuged at 1000 rpm for 1 minute in a refrigerated centrifuge (2 – 8^{O}C). The microtitre plate was placed into the holder in the ABI One Step Plus Real-time PCR machine. The manufacturer's instruction was followed in setting up the template –*S. pneumoniae* genotyping. To commence testing, SYBR Chemistry and Standard mode, with Absolute Quantification, were selected. The run was started and saved correctly. The thermal profile comprised of initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 5s, 60°C for

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30s, 72°C for 10s, and a final melt cycle of 72°C to 95°C at ramp rate of 0.3°C/s.

l) Result Analysis

After the run, the amplification curve and cycle threshold (Ct) values were inspected. Ct values of < 35 were positive; Ct values of 35 – 40 were equivocal; Ct values > 40 were negative. For the equivocal ones, the amplification curve and melting curve should be checked to decide the result. If it melts at the same specific temperature as the positive cases, it should be considered positive.

m) Sequenced Results from MLST

The genome sequences obtained from genotyping (MLST protocol) was uploaded to the publicly available Streptococcus PubMLST.org database(http://pubmlst.org/streptococcuspneumoniae) [Jolley & Maiden, 2014], powered by the Bacterial Isolate Genome Sequence Database (BIGSdb) software [Jolley & Maiden, 2010] for the determination of sequence type (ST), biotype, and global epidemiology status.

n) Extracting Typing Information from a Local Genome File

The MLST scheme from a drop-down box of the PubMLST.org database for Streptococcus was selected, and the analysis run started by clicking the submit button. Individual allelic matches identified along with the sequence type (ST) if the combination of alleles has been previously defined [Jolley et al., 2018]. Typing information can be readily extracted from whole genome sequence assemblies using the sequence query pages. Genome assembly contids pasted into the sequence query form of the database, and the required scheme or locus was selected. Any locus exact matches are displayed, and, if this corresponds to a defined combination of alleles, the profile definition (Sequence type (ST) / clonal complex (cc) for MLST) was displayed [Jolley et al., 2018].

III. Results

Of the eight genotyped isolates with MLST, three (37.5 %) had detectable genotypes, while five (62.5 %) were undetectable genotype strains. Of the seven genotypes tested, we encountered only two genotypes: genotype *aroE,* two cases (25 %) found circulating within serotype 4 (*Wzy* 4), and genotype *gki* in one case (12.5 %) found circulating within serotype 5 (*Wzy* 5) (Table 2). For genotype *aroE,* one sequence type (ST) 12750 was identified, while for genotype *gki,* two sequence types (ST) 11337 and 13103 were identified (Table 3). Results for *S. pneumoniae* genotypes sequence profile extracted from PubMLST.org powered by BIGSdb software as shown in Tables 3 to 5.

IV. Discussion

Knowledge of the genetic diversity of pathogens is being exploited more directly in the study of epidemiology. In molecular epidemiology parlance, genetic diversity is commonly referred to as 'typing' or 'genotyping'. Genotyping is being practiced widely in medical microbiology and has shown to be an invaluable tool in tracking strains responsible for disease outbreaks; particularly useful in studying and controlling nosocomial outbreaks and to ascertain whether the relapse of an infectious disease after therapeutic intervention, was due to treatment failure or recolonization of the host by a new strain [Virdi & Sachdeva, 2005]. *S. pneumoniae* genome sequence on the PubMLST.org for typing by MLST allelic profile through a search by specific schemes such as Penicillin-binding proteins (PBPs) and Pneumococcal surface protein A (PspA), results were not available. However, under the PubMLST pneumococcal genome library page, we could extract the global epidemiology status.

Our query of genotype *aroE* by filtering on serotype 4 (*Wzy* 4) revealed varying sequence types (ST) and alleles. While isolates from The Netherlands had two STs (ST 247, allele 16 and ST 205, allele 10), those from South Africa had the same ST and allele (ST 1221, allele 7) [Gladstone et al., 2020]. Though they were silent in the genomic status of these isolates; our search from the PubMLST.org database confirmed that serotype 4 (*Wzy* 4) was also found in these countries (Table 4). Our query of genotype *gki* by filtering on serotype 5 (*Wzy* 5) displayed a single ST 5659 and allele number 16 from South Africa alone submitted between 2006 and 2012 (Table 5) [Gladstone et al., 2020].

V. Conclusion

The MLST results provided an overview of circulating *S. pneumoniae* genotypes and their genetic diversity status, which is a piece of information for public health strategies such as vaccination. To develop more effective vaccines, it is imperative that the candidate vaccines must be evaluated against a set of carefully selected genotypes, which are representative of the pathogen population [Dykhuizen et al., 1993]. The tracking of identified genotypes of *S. pneumoniae* (*aroE* and *gki*) to a pneumococcal meningitis outbreak in parts of Northern Nigeria is being reported as baseline data for reference.

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Table 1: *S. pneumoniae* MLST scheme, including gene locus, amplicon length, and trimmed length of sequence used for allelic determination on http://www.mlst.net platform (CDC, 2011, Chap. 12).

Housekeeping genes	Gene locus	Trimmed length
Shikimate dehydrogenase	*aroE*	405
Glucose-6-phosphate dehydrogenase	*gdh*	460
Glucose kinase	*gki*	483
Transketolase	*recP*	450
Signal peptidase I	*spi*	474
Xanthine phosphoribosyltransferase	*xpt*	486
D-alanine-D-alanine ligase	*ddl*	441

Table 2: Prevalence of circulating *S. pneumoniae* genotypes amongst the various identified serotypes in parts of Northern Nigeria

Gene locus	Allele	Number encountered	%	Circulating amongst serotype	Number of serotype
aroE	405	2	25.0	*Wzy 4*	1
gki	483	1	12.5	*Wzy 5*	1
Undetectable genotype strains		5	62.5		
Total		8	100.0		

Gene locus - Housekeeping genes
aroE- Shikimate dehydrogenase
gki- Glucose kinase

Table 3: Genotypes of *S. pneumoniae* allelic profile for Sequence type (ST) and clonal complex (cc) extracted from PubMLST.org powered by BISGdb software

Gene Locus	Allele	Sequence Type (ST)	Clonal complex (cc)	BURST analysis Singletons	
				ST	Frequency
aroE	405	12750	Nil	Nil	
gki	483	11337	Nil	11337	1
		13103	Nil	13103	1

Table 4: *S. pneumoniae* genotype *aroE* sequence profile extracted for Serotype 4 (*Wzy 4*) from PubMLST.org powered by BIGSdb for global epidemiology status

Id	Isolate	Country	Year	Serotype	MLST (*aroE*) Allele	ST
49181	AMCSP09	The Netherlands	2008	4	16	247
49190	AMCSP18	The Netherlands	2008	4	10	205
116970	GPS_ZA_3068	South Africa	2014	4	7	1221
117961	GPS_ZA_1432	South Africa	2008	4	7	1221

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Table 5: *S. pneumoniae* genotype *gki* sequence profile extracted for Serotype 5 (*Wzy 5*) from PubMLST.org powered by BIGSdb for global epidemiology status

Id	Isolate	Country	Year	Serotype	MLST (*gki*) Allele	ST
117626	SA_GPS_SP259	South Africa	2012	5	16	5659
117672	SA_GPS_SP191	South Africa	2012	5	16	5659
117879	GPS_ZA_695	South Africa	2006	5	16	5659
117908	GPS_ZA_1145	South Africa	2010	5	16	5659
118148	GPS_ZA_1806	South Africa	2010	5	16	5659
118188	GPS_ZA_2259	South Africa	2011	5	16	5659

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Determinação Do Perfil De Prescrição De Oxandrolona E Estanazolol Em Farmácias De Manipulação De Vitória Da Conquista No Ano De 2019

By Pedro Augusto Cruz Correia & Matheus Santos Marques

Abstract- Anabolic androgenic steroids (AAS) are synthetic substances produced from the hormone testosterone. Among some of the AASs used, are the substances oxandrolone and stanazolol, which can be prescribed in order to obtain results in sports or for therapeutic purposes. Although they demonstrate benefits, the great concern regarding the use of these substances is due to the large number of possible adverse effects. With that said, the present research was formulated with the intention of making a study about the use of anabolic steroids in manipulation pharmacies, and to determine its use profile, the pharmaceutical form and the dosage, to identify the reasons that lead to the use of steroids, and identify the prescriber's specialization and relate it to its use.

Keywords: *anabolic steroids. specialty. prescribers.*

GJMR-C Classification: *DDC Code: 362.29088796 LCC Code: RC1230*



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Determinação Do Perfil De Prescrição De Oxandrolona E Estanazolol Em Farmácias De Manipulação De Vitória Da Conquista No Ano De 2019

Pedro Augusto Cruz Correia [α] & Matheus Santos Marques [σ]

Resumo- Os esteroides anabolizantes androgênicos (EAA) são substâncias sintéticas produzidas a partir do hormônio testosterona. Entre alguns dos EAAs utilizados, estão as substâncias oxandrolona e estanazolol, que podem ser prescritas com intuito de obter resultados no meio desportivo ou para fins terapêuticos. Embora demonstrem benefícios, a grande preocupação em relação ao uso destas substâncias se deve à grande quantidade de efeitos adversos possíveis. Diante disso, a presente pesquisa foi formulada com o intuito de fazer um estudo a respeito do uso de esteroides anabolizantes em farmácias de manipulação, e determinar o seu perfil de uso, a forma farmacêutica e a dosagem, identificar os motivos que levam ao uso dos esteroides, e identificar a especialização do prescritor e relacionar ao seu uso. A pesquisa foi realizada na maior rede de farmácias de manipulação da cidade de Vitória da Conquista, onde foram coletadas todas as ordens de manipulação que continham as substâncias oxandrolona e estanazolol durante o período do ano de 2019, e foi encontrado um total de 542 ordens de manipulação, sendo que a oxandrolona foi muito mais utilizada que o estanazolol, 58,3% dos pacientes pertenciam ao sexo feminino, e 84,9% dos médicos prescritores foram generalistas. A forma mais utilizada foi em cápsulas por via oral, e a dosagem mais frequente foi a de 10mg para as duas drogas. A grande quantidade de médicos generalistas que prescrevem anabolizantes mostrou-se um dado preocupante, devido aos diversos problemas acarretados quando ocorre o uso dessas drogas de forma desnecessária. O conhecimento sobre anabolizantes é de extrema importância para a prevenção do seu uso indevido, por isso espera-se que profissionais especializados só utilizem tais substâncias em casos de terapia onde são de fato necessárias.

Palavras-Chave: esteroides anabolizantes. especialidade. prescritores.

Abstract- Anabolic androgenic steroids (AAS) are synthetic substances produced from the hormone testosterone. Among some of the AASs used, are the substances oxandrolone and stanazolol, which can be prescribed in order to obtain results in sports or for therapeutic purposes. Although they demonstrate benefits, the great concern regarding the use of these substances is due to the large number of possible adverse effects. With that said, the present research was formulated with the intention of making a study about the use of anabolic steroids in manipulation pharmacies, and to determine its use profile, the pharmaceutical form and the dosage, to identify the reasons that lead to the use of steroids, and identify the prescriber's specialization and relate it to its use. The research was carried out in the largest network of manipulation pharmacies in the city of Vitória da Conquista, where all manipulation orders containing the substances oxandrolone and stanazolol were collected during the period of the year 2019, and a total of 542 manipulation orders were found, with oxandrolone being much more used than stanazolol, 58.3% of patients were female, and 84.9% of prescribers were generalists. The most used form was in oral capsules, and the most frequent dosage was 10mg for both drugs. The large number of general physicians who prescribe anabolic steroids proved to be a worrying fact, due to the various problems caused when these drugs are used unnecessarily. Knowledge about anabolic steroids is extremely important to prevent their misuse, and so it is expected that specialized physicians will only use these substances in cases of therapy where they are really necessary.

Keywords: anabolic steroids. specialty. prescribers.

I. Introdução

Os esteroides anabolizantes androgênicos (EAA) são substâncias sintéticas produzidas a partir do hormônio testosterona. Essas substâncias foram inicialmente sintetizadas para diversos fins terapêuticos, mas devido ao fato de possuírem efeitos sobre o aumento da síntese proteica e a melhora nas reservas de energia e recuperação musculares após treinamento físico, passaram a ser utilizados por atletas para melhorar o desempenho esportivo (LIMA, et al, 2015). Essas drogas proporcionam a melhoria do desempenho durante exercícios físicos, com aumento de massa muscular, perda de gordura e também agem na melhora em questões estéticas. (MARKOVICZ, 2019). Entre alguns dos EAAs utilizados, estão as substâncias oxandrolona e estanazolol, moléculas modificadas a partir da testosterona, que podem ser prescritas com intuito de obter resultados no meio desportivo ou para fins terapêuticos (MARKOVICZ, 2019). Embora demonstrem benefícios, a grande

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preocupação em relação ao uso destas substâncias se deve à grande quantidade de efeitos adversos possíveis, em diversos órgãos e sistemas, além dos efeitos psicológicos, incluindo mudanças de humor, comportamento agressivo, depressão, hostilidade e surtos psicóticos (LIMA, et al, 2015).

Diante de tais afirmações, a presente pesquisa foi formulada com o intuito de fazer um estudo a respeito do uso de esteroides anabolizantes em farmácias de manipulação, onde estas drogas só podem ser obtidas por meio legal e documentado. A pesquisa traz impacto para os usuários de esteroides anabolizantes ao voltar a atenção aos seus diversos riscos, devido à grande quantidade de efeitos adversos provocados por essas drogas, e se mostra oportuna nesse momento visto que o consumo no Brasil demonstra ser elevado, acreditando-se estar concentrado em jovens do sexo masculino com faixa etária de 18-34 anos, englobando praticantes de esporte, principalmente musculação, sendo que no Brasil, 8-55% dos frequentadores de academia fazem uso de anabolizantes (FARIA, et al, 2015). O estudo desse tema mostra-se relevante para a área de farmácia pois permite avaliar a necessidade do uso dessas drogas e se não existem outras alternativas terapêuticas menos danosas para o alcance do objetivo do paciente.

As farmácias pesquisadas fazem parte da maior rede da cidade, que contém uma matriz e quatro filiais, distribuídas nos dois grandes lados da cidade (oeste e leste), próxima dos grandes centros médicos e hospitais e com elevada demanda de produtos. Os EAA disponíveis para venda nas farmácias em questão são a oxandrolona e estanazolol, e a partir das prescrições contendo essas drogas pretende-se determinar o seu perfil de uso, a forma farmacêutica e a dosagem, identificar os motivos que levam ao uso dos esteroides, e identificar a especialização do prescritor e relacionar ao seu uso.

II. Metodologia

A pesquisa foi realizada na maior rede de farmácias de manipulação da cidade de Vitória da Conquista, que conta com cinco farmácias, onde foram coletadas todas as ordens de manipulação que contêm as substâncias oxandrolona e estanazolol durante o período de um ano, entre os meses janeiro a dezembro de 2019.

A coleta de dados foi realizada de forma digital, por meio da obtenção de arquivos contendo as ordens de manipulação de oxandrolona e estanazolol de cada uma das farmácias, seguida do armazenamento em pen-drive e computador para posterior leitura e tabulação dos dados. Uma ordem de manipulação é uma ficha impressa que determina as quantidades de matéria prima, embalagem, excipientes, entre outras informações relevantes que identifiquem e caracterizem o procedimento a ser realizado numa formulação. A amostra estudada se tratou de usuários destas substâncias.

Os dados coletados a partir das ordens de manipulação englobam o perfil dos usuários, sendo que entre as informações estavam o sexo dos pacientes, número de registro no conselho regional de medicina (CRM) do médico, forma farmacêutica e dosagem da substância. O CRM de cada médico foi o dado utilizado para encontrar as especialidades dos médicos pesquisados. Isso foi possível já que todo profissional formado em medicina deve ter registro no conselho federal de medicina (CFM), que é o órgão por fiscalizar e normatizar a prática médica. O CFM possui um website onde há uma aba que permite ao cidadão comum realizar a pesquisa por qualquer médico utilizando o seu CRM, que então informa a especialidade do médico. Dessa forma, houve o intuito de utilizar esses dados para identificar qual especialidade médica é responsável pelo maior número de prescrições de esteroides anabolizantes na farmácia de manipulação, bem como discutir as possíveis finalidades do uso da droga.

Após a obtenção dos dados, utilizou-se o programa de análise estatística SPSS para realizar a organização e formar tabelas com os mesmos.

III. Resultados e Discussão

Foi encontrado um total de 542 ordens de manipulação contendo oxandrolona e estanazolol ao longo do período de 2019, sendo que a maioria dos pacientes que usaram a substância foram do sexo feminino. A tabela 1 a seguir mostra a frequência de prescrição das drogas:

Tabela 1: Frequência de prescrições dos medicamentos EAA presentes nas farmácias de manipulação estudadas em 2019

Medicamento	Frequência de prescrições	Porcentagem
Oxandrolona	447	82,5%
Estanazolol	92	17%
Ambos	3	0,6%
Total	**542**	**100%**

Fonte: autor

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Em relação aos medicamentos escolhidos, a oxandrolona foi muito mais utilizada que o estanazolol, e durante o ano houveram apenas três receitas onde esses medicamentos foram prescritos de forma associada. Em relação ao sexo dos pacientes, 316 (58,3%) pertenciam ao sexo feminino e 226 (41,7%) pertenciam ao sexo masculino. O mecanismo de ação da testosterona e dos androgênios são divididos em duas categorias: efeitos androgênicos, associados à função reprodutora e às características sexuais secundárias masculinas, e efeitos anabólicos, causadores da estimulação do crescimento e a manutenção dos tecidos não reprodutores. Ambos os mecanismos agem em um único receptor, sendo o tecido alvo o fator determinante da resposta hormonal (ANDRADE, 2016).

O uso maior de oxandrolona pode se dar devido ao fato de que, segundo Fonini (2006), a oxandrolona causa menos efeitos colaterais pronunciados. Isso também teria relação com a maior presença de pacientes do sexo feminino, já que devido à menor intensidade de efeitos adversos pronunciados essa droga termina sendo um dos EAA mais escolhidos para mulheres.

O estudo de Bolognia (2000) corrobora com esse fato, onde foi realizado o tratamento de uma paciente com lipodermatoesclerose, uma patologia dermatológica, com estanazolol e foi percebido que a droga alterou os níveis de enzimas hepáticas, sugerindo possível hepatotoxicidade. Ao se realizar a substituição pela oxandrolona, a paciente apresentou melhora e os indicadores hepáticos voltaram ao normal. Diferentemente da maioria dos EAA utilizados por via oral, a oxandrolona é associada a uma baixa hepatotoxicidade pois passa por um metabolismo hepático limitado (BOLOGNIA, 2000).

Entretanto, outro estudo realizado numa paciente que também sofria da mesma doença relatou que o uso de estanazolol no tratamento ocorreu sem a presença de efeitos adversos significativos. No meio do tratamento o uso do estanazolol foi interrompido devido à indisponibilidade da droga no mercado, quando então ocorreu a substituição dessa droga pela oxandrolona, que continuou por um longo período e também sem a presença de efeitos adversos significativos. Mesmo assim, os autores notificaram que caso surgissem indicativos de hepatotoxicidade no tratamento da lipodermatoesclerose, a substituição do estanazolol pela oxandrolona seria uma boa estratégia (GOMES, 2019). Como o número de prescrições de estanazolol foi muito mais baixo do que o de oxandrolona, um possível viés a se considerar seria a falta de abastecimento da droga no mercado para a farmácia, como ocorreu no caso do estudo de GOMES (2019).

Em relação às prescrições associadas às especialidades dos médicos, obteve-se os seguintes dados, representados na tabela 2:

Tabela 2: Frequência das especialidades médicas prescritoras de EAA nas farmácias de manipulação estudadas durante o ano de 2019

Especialidade médica	Frequência deprescrições	Porcentagem
Não registrada	460	84,9%
Anestesiologia	1	0,2%
Angiologia	3	0,6%
Cardiologia	2	0,4%
Cirurgia geral	9	1,7%
Clínica médica	1	0,2%
Dermatologia	3	0,6%
Endocrinologia	6	1,1%
Gastroenterologia	5	0,9%
Ginecologia e Obstetrícia	5	0,9%
Hematologia	1	0,2%
Medicina do trabalho	1	0,2%
Medicina do tráfego	3	0,6%
Neurocirurgia	4	0,7%
Oftalmologia	3	0,6%
Ortopedia	4	0,7%
Pediatria	6	1,1%
Psiquiatria	4	0,7%
Radiologia	9	1,7%
Urologia	12	2,2%

Fonte: autor

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A partir dos dados coletados, percebe-se que a grande maioria dos médicos prescritores (84,9%) são generalistas, não possuindo especialização registrada no CFM em qualquer área que os tornem mais capacitados para realizar a prescrição segura dos esteroides anabolizantes, sejam esses para o uso terapêutico ou para o uso desportivo. A associação entre as especialidades e possíveis usos das drogas será discutida mais a frente com a tabela 3.

Dos médicos especialistas, a maioria tem especialidade em urologia, seguido de cirurgia geral e radiologia, depois por endocrinologia e pediatria. Os EAA também estavam acompanhados a diversas drogas associadas em várias prescrições, incluindo por exemplo antioxidantes e antineoplásicos, mas a maioria se tratava de suplementos dietéticos, aminoácidos, e substâncias que auxiliam em perda de peso ou estímulo do apetite, o que indica um perfil de uso com fins estéticos e/ou desportivos.

Já no caso de utilizações terapêuticas, os esteroides podem auxiliar no tratamento de diversos problemas, como osteoporose, disfunções de crescimento, tratamento de queimaduras graves (MARKOVICZ, 2019), patologias em que há déficit de testosterona, balanço proteico negativo, câncer de mama, angiodema hereditário, anemia aplástica, endometriose grave, estímulo do crescimento em caso de puberdade masculina tardia, insuficiência renal aguda e mielofibrose (FARIA, et al, 2015).

Dessa forma, percebe-se que algumas das especialidades presentes justificam o uso dos EAA para utilizações terapêuticas. Segundo Markovicz (2019) e Faria (2015), essas drogas podem ser utilizadas no tratamento de diversas patologias, que podem então ser associadas com as especialidades prescritoras, conforme demonstra a tabela 3:

Tabela 3: Relação entre especialidades médicas e doenças tratadas com EAA

Especialidade médica	Patologias
Ortopedia, Endocrinologia	Osteoporose
Endocrinologia, Pediatria, Clínica médica	Disfunções de crescimento
Dermatologia, Cirurgia geral	Tratamento de queimaduras graves
Endocrinologia, Urologia, Ginecologia, Clínica médica	Déficit de testosterona
Endocrinologia, Clínica médica	Balanço proteico negativo
Ginecologia	Câncer de mama
Angiologia, Clínica médica	Angiodema hereditário
Hematologia	Anemia aplástica
Ginecologia	Endometriose
Endocrinologia, Clínica médica	Estímulo do crescimento em puberdade masculina tardia
Urologia, Clínica médica	Insuficiência renal aguda
Neurocirurgia	Mielofibrose

Fonte: autor

Caso os profissionais em questão tenham realizado a prescrição com intuito terapêutico, ainda assim percebe-se que as especialidades anestesiologia, cardiologia, gastroenterologia, medicina do trabalho e do tráfego, oftalmologia e psiquiatria não tem correlação com o uso terapêutico das substâncias em estudo.

A grande quantidade de prescrições realizadas sem a especialidade capacitada para tal é um dado preocupante, visto que os EAA provocam diversos efeitos negativos à saúde. Os efeitos colaterais estão presentes em quase 100% dos usuários, sendo os mais comuns a acne, atrofia testicular, retenção hídrica, alterações do humor e ginecomastia. Além disso, há alterações hormonais, enzimáticas, em células do sistema hematopoiético e no perfil lipídico sanguíneo (LIMA, et al, 2015). Nas mulheres destaca-se o crescimento de pelos, voz grave, diminuição dos seios, além do aumento do clitóris e ausência do ciclo menstrual (FARIA, et al, 2015).

Entre os efeitos colaterais agudos estão as dores de cabeça, retenção de líquidos, irritação gastrointestinal, diarréia, dores de estômago, pele oleosa, icterícia, alterações menstruais e hipertensão. Com a administração de esteroides exógenos, ocorre também a redução da secreção de esteroides endógenos. Nos homens essa supressão endócrina pode levar a hipogonadismo com alterações na função sexual, sendo um estado reversível após a descontinuação do uso. O uso dessas substâncias também está fortemente associado a danos celulares no miocárdio, pois aumentam a resistência vascular periférica e levam à hipertrofia cardíaca juntamente com a diminuição da contratilidade do coração. Os esteroides também possuem efeitos sobre a função tireóidea, com a diminuição de proteínas responsáveis pela manutenção das concentrações séricas de hormônios tireoideanos (LIMA, et al, 2015).

Outros efeitos são a retenção iônica, icterícia e tumores no fígado, aumento das lesões nas

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articulações (por não estarem aptas ao crescimento exagerado da musculatura), tremores, retenção de líquido, agravamento da apnéia do sono e estrias, oligúria ou disúria, e aumento da próstata. A interrupção abrupta do uso destas substâncias pode levar à depressão por abstinência (FARIA, et al, 2015).

Em relação às formas farmacêuticas mais utilizadas, obteve-se os dados mostrados na tabela 4:

Tabela 4: Frequência de formas farmacêuticas aplicadas aos EAA nas farmácias de manipulação estudadas durante o ano de 2019

Forma farmacêutica	Frequência prescrita	Porcentagem
Cápsula (oral)	415	76,6%
Resvin (oral)	21	3,9%
Pentravan (transdérmico)	8	1,5%
Versapro (transdérmico)	63	11,6%
Sorbitol (oral)	22	4,1%
Gotas sublingual (oral)	13	2,4%
Total	542	100%

Fonte: Autor

As formas farmacêuticas de escolha englobaram administração por via oral e por via transdérmica, sendo que a forma mais utilizada foi em cápsulas por via oral, seguida pela base transdérmica versapro.

As formas mais conhecidas dos EAA são as orais e injetáveis, sendo que as orais sofrem metabolismo de primeira passagem pelo fígado e têm menor tempo de circulação no sistema, sendo seu excesso eliminado na urina. Já as injetáveis possuem um maior tempo de vida por não sofrer esta ação do fígado (ANDRADE, 2016). Como exemplo, a testosterona natural, quando administrada como medicamento por via oral, torna-se pouco eficaz devido ao rápido metabolismo hepático. Sua meia vida está em aproximadamente vinte minutos e cerca de 90% de seus metabólitos são excretados na urina (ANDRADE, 2016).

Dessa forma, segundo Andrade (2016), oxandrolona e estanazolol são drogas ideais para a administração por via oral pois são modificadas para aumentar sua resistência ao metabolismo hepático. Elas são classificadas como 17 α-derivados devido à adição de um grupo alquila à posição 17α da testosterona, o que retarda o seu catabolismo hepático, proporciona melhor afinidade ao receptor e dificuldade de ser convertido em estradiol, mas também causa uma hepatotoxicidade não observada na testosterona natural. (ANDRADE, 2016; PEREIRA, 2019).

Já a forma de uso por via transdérmica, onde foram utilizados os veículos versapro e pentravan, fornece concentrações de testosterona mais estáveis do que o uso de injeções com ésteres de testosterona (PEREIRA, 2019) e constitui outra estratégia para reduzir o metabolismo hepático de primeira passagem.

Em relação às dosagens dos medicamentos, obteve-se os dados mostrados na tabela 5, sendo que a oxandrolona apresentou prescrições com concentrações em miligramas e porcentagem, e o estanazolol apenas em miligramas:

Tabela 5: Frequência de dosagens prescritas de EAA nas farmácias de manipulação estudadas durante o ano de 2019

Dosagem da oxandrolona em mg	Frequência de prescrições	Dosagem da oxandrolona em %	Frequência de prescrições	Dosagem do estanazolol	Frequência de prescrições
0,5 mg	2	0,5%	3	3 mg	2
2,5 mg	9	0,6%	7	4 mg	2
3 mg	12	0,7%	37	5 mg	15
4 mg	4	0,8%	10	7 mg	7
5 mg	53	0,9%	5	10 mg	48
7 mg	6	1%	3	12 mg	1
7,5 mg	3	1,5%	1	15 mg	15
8 mg	8			20 mg	4
9 mg	2			30 mg	1
10 mg	151				
15 mg	13				
20 mg	116				
30 mg	5				
Total	**384**		**66**		**95**

Fonte: autor

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Para a oxandrolona, a dosagem mais frequentemente utilizada é a de 10mg, havendo uma média de dosagem prescrita de 9,3 para as doses em miligramas e 0,857 para as em porcentagem. Para o estanazolol, a dosagem mais frequente também é a de 10mg, e obteve-se uma média de dose prescrita de 11,777mg.

Segundo Fonini (2006), a meia-vida do estanazolol injetável é de 1 dia, e a de uso oral é de 9 horas. É um esteroide que, em alta dosagem, pode apresentar mais toxicidade ao fígado, principalmente na forma oral. A dosagem indicada é de 16 a 30 mg/dia na forma oral para homens e de 4 a 8 mg/dia para mulheres. A oxandrolona também possui pequena meia-vida de 9 horas, e tem baixa concentração por comprimido (entre 2,5 ou 5mg), sendo que seu uso varia entre 4 a 17 comprimidos por dia para homens e 2 a 4 comprimidos por dia para mulheres (FONINI, 2006). Bolognia (2000) e Gomes (2019) relataram o uso terapêutico de oxandrolona 10mg 2 vezes por dia e estanazolol 2 mg uma vez e duas vezes por dia.

Segundo Markovicz (2019), existem três formas de se utilizar os diferentes esteroides, que podem ser ciclo, pirâmide e stacking. O ciclo se define por qualquer período de utilização da droga, podendo variar de 4 a 18 semanas, sendo feito de tempos em tempos e com intervalo entre eles; a pirâmide se caracteriza pelo início com dosagens pequenas e aumento gradativo até a sua saturação e então redução regressiva até encerrar- se o período; por fim, o stacking se refere ao uso de várias drogas ao mesmo tempo de acordo com a sua toxicidade.

As ordens de manipulação coletadas para a presente pesquisa não trazem a informação da posologia, mas pode-se observar que as doses presentes variam bastante, contendo até altas concentrações de 30mg, e muitas amostras de 20mg no caso da oxandrolona. Segundo Markovicz (2019), para se obter alterações corporais e esportivas, as doses utilizadas geralmente tendem a ser muito maiores do que as recomendadas. Isso pode ter relação com algumas destas altas dosagens encontradas, indicando uso indevido, ou sem fins terapêuticos.

IV. Considerações Finais

Através dessa pesquisa, os objetivos propostos foram alcançados, obtendo-se um perfil de uso com uma grande quantidade de médicos prescritores não especializados, havendo muitas associações a substâncias que atuam como suplementos dietéticos, indicando uso para fins estéticos/esportivos, com dosagens altas em alguns casos e em seis formas farmacêuticas diferentes.

A grande quantidade de médicos generalistas que prescrevem anabolizantes mostra-se um dado preocupante, devido aos diversos problemas acarretados quando ocorre o uso dessas drogas de forma desnecessária. O conhecimento sobre anabolizantes e suas ações no organismo é de extrema importância para a prevenção do seu uso indevido, por isso espera-se que profissionais especializados, com conhecimento mais aprofundado acerca do assunto, só realmente utilizem essas substâncias, com tamanho potencial de causar danos, em casos de terapia onde são de fato necessárias, reduzindo a quantidade de prescrições contendo as mesmas.

A informação e o aconselhamento sobre o uso de EAA deve ser difundida, sendo interessante realizar estratégias sobre a difusão do conhecimento a respeito destas drogas para a população, e no caso da rede de farmácias de manipulação, os próprios farmacêuticos podem tomar o papel de informar os pacientes ao realizar a atenção farmacêutica adequada, pois a partir dessa abordagem em pesquisa pode-se obter esclarecimento em relação ao uso destas substâncias, proporcionando melhor orientação ao paciente por parte do farmacêutico.

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Invasive Genotypes of *Haemophilus Influenzae* Strains Implicated with Cerebrospinal Meningitis Outbreak in Parts of Northern Nigeria

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Abstract- The genetic profile of a given strain of bacteria generated by a specific genotyping method can be as unique as a fingerprint. Information on the circulating invasive genotypes of *Haemophilus influenzae* in Northern Nigeria is unavailable in the literature. We aimed at determining the invasive genotypes of *H. influenzae* strains implicated with cerebrospinal meningitis outbreak in parts of Northern Nigeria. The multilocus sequence typing scheme, a DNA sequencing-based genotyping method of sequencing segments of seven internal housekeeping genes was used. The PubMLST.org database and Bacterial Isolate Genome Sequence Database software was equally used.

Keywords: *bacterial genotypes, genetic profile, serotypes, H. influenzae, CSM, outbreak, northern Nigeria.*

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Invasive Genotypes of *Haemophilus Influenzae* Strains Implicated with Cerebrospinal Meningitis Outbreak in Parts of Northern Nigeria

Iseimokumo Christopher Peletiri [α], Eugene Ifeanyichukwu Ikeh [σ] & Grace Mebi Ayanbimpe [ρ]

Abstract- The genetic profile of a given strain of bacteria generated by a specific genotyping method can be as unique as a fingerprint. Information on the circulating invasive genotypes of *Haemophilus influenzae* in Northern Nigeria is unavailable in the literature. We aimed at determining the invasive genotypes of *H. influenzae* strains implicated with cerebrospinal meningitis outbreak in parts of Northern Nigeria. The multilocus sequence typing scheme, a DNA sequencing-based genotyping method of sequencing segments of seven internal housekeeping genes was used. The PubMLST.org database and Bacterial Isolate Genome Sequence Database software was equally used. Of the 12 genotyped isolates, six (50 %) had detectable genotypes: genotype *adk* in two cases (16.7 %) found circulating amongst serotypes e (*ecsH*) and f (*bexD*); genotype *fucK*, two cases (16.7 %) amongst serotypes b (*bcsB*) and f (*bexD*); and genotype *mdh* in two cases (16.7 %) amongst serotypes b (*bcsB*) and e (*ecsH*). The three genotypes are now documented as invasive strains. Therefore, for a preventive vaccination programme against cerebrospinal meningitis caused by *H. influenzae* to be successful in Nigeria, these identified invasive genotype strains should be included in the composition of the vaccines for administration.

Keywords: *bacterial genotypes, genetic profile, serotypes, H. influenzae, CSM, outbreak, northern Nigeria.*

I. Introduction

Haemophilus influenzae is a Gram-negative, pleomorphic coccobacillus responsible for life-threatening invasive diseases such as septicemia and bacterial meningitis in young children [Peltola, 2000]. *H. influenzae* may be encapsulated (typable) with one of six polysaccharide capsules designated type Hi a *(acsB)*, b *(bcsB)*, c *(ccsD)*, d *(dcsE)*, e *(ecsH)*, and f *(bexD)* or unencapsulated (non-typable *H. influenzae*, NTHI). Beyond the newborn period, *H. influenzae* is one of the most common cause of bacterial meningitis [CDC, 2011, Chap. 2]. Of the infections caused by *H. influenzae,* meningitis is of particular importance because of its potential to cause lasting neurological damage, even with appropriate supportive and antibiotic treatment (Rodrigues et al., 1971). Bacterial meningitis is a life-threatening condition that has remained a serious global health problem [CDC, 2011, Chap. 1 & 2].

In a very recent study by Peletiri et al., (2021b), *H. influenzae* was reported as the second major aetiology of bacterial meningitis in parts of Northern Nigeria. In that same report, the authors reported the varying circulating *H. influenzae* serotypes. However, information on the circulating genotypes of *H. influenzae* in Northern Nigeria is unavailable in the literature. The genetic profile of a given strain generated by a specific genotyping method can be as unique as a fingerprint [Wenjun et al., 2009].

The multilocus sequence typing (MLST) protocol is a DNA sequencing-based genotyping method that generates the original sequence of nucleotides and discriminates among bacterial strains directly from polymorphisms in their DNA, and has been developed with a global epidemiology perspective [Chan et al., 2001]. MLST indexes the sequences of seven internal housekeeping gene fragments to identify bacterial genotypes and associate them with biological properties [Chan et al., 2001; Jolley et al., 2004; Maiden et al., 1998]. MLST scheme is available for *H. influenzae* based on DNA sequencing of seven housekeeping enzyme genes (adk, atpG, frdB, fucK, mdh, pgi, and recA) for characterization of capsulated and uncapsulated *H. influenzae* isolates, metagenomic DNA (mDNA) or genomic DNA (gDNA) extracts [Meats et al., 2003]. We aimed at determining the invasive genotypes of *H. influenzae* strains implicated with cerebrospinal meningitis outbreak in parts of Northern Nigeria.

II. Materials and Methods

a) Ethics Approval

Ethical approval was obtained from the Health Research Ethics Committees of National Hospital, Abuja, Nigeria (NHA/EC/034/2015); Federal Capital Development Authority Health Services, Abuja, Nigeria (FHREC/2017/01/27/03-04- 17); Kebbi State Ministry of Health, Nigeria (MOH/KSREC/VOL.1/56/No101.3/2015); Plateau State Ministry of Health, Nigeria (MOH/MIS/202/VOL.T/X, 2017); Sokoto State Ministry of Health, Nigeria (SMH/1580/V.IV, 2017); and Zamfara State Ministry of Health, Nigeria (ZSH REC/ 02/03/2017) [Peletiri et al., 2021a; 2021b].

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b) Consent to Participate

Written informed consent for the storage and future use of the unused sample, and sample material and data transfer agreement were also obtained [Peletiri et al., 2021a; 2021b].

c) Sample Size Determination

The sample size was calculated using the Cochran formula [Cochran, 1977] for calculating simple proportion. At 0.05 alpha level of significance, 95 % confidence level and patient population size of seventy-seven and a previous prevalence of 13.7 %, a sample size of 181.7, which was adjusted to 210 samples after calculating 10 % attrition [Peletiri et al., 2021a; 2021b]. The subjects were recruited consecutively until the sample size was attained [Peletiri et al., 2021a; 2021b].

d) Sample Collection

The collection of cerebrospinal fluid (CSF) samples were as reported previously [Peletiri et al., 2021a].

e) Extraction and Quality Check of Metagenomic DNA

The metagenomic DNA extraction protocol and quality check was as previously reported by Peletiri and colleagues [Peletiri et al., 2021a].

f) Multiplex Real-Time PCR for H. influenzae Detection

The molecular detection of *H. influenzae* with multiplex Real-time PCR protocol was as previously reported [Peletiri et al., 2021b].

g) Singleplex Real-time PCR for H. influenzae Characterization

The molecular characterization of *H. influenzae* with singleplex Real-time PCR protocol was as previously reported [Peletiri et al., 2021b].

h) Sample Selection

Of the appropriately characterized 26 *H. influenzae* serotyped strains with singleplex Real- time PCR as reported by Peletiri and co-authors [Peletiri et al., 2021b], 12 (46.2 %) were properly selected for genotyping to ensure both geographical coverage (spread) and serotype representation or distribution.

i) Ultilocus Sequence Typing (MLST) Protocol for H. influenzae Genotyping

The Real-time PCR method of Multilocus Sequence Typing (MLST) using SYBR chemistry, is designed to genotype selected genotypic markers of *H. influenzae*. The assay detects seven genotypic markers: adk, atpG, frdB, fucK, mdh, pgi, and recA [Maiden, 2000; Maiden et al., 1998; Meats et al., 2003], and as in Table 1. The primers used for amplification by Real-time PCR were: adk-F (5'-GGTGCACC-GGGTGCAGGTAA-3'), and adk-R (5'-CCTAAGA-TTTTATCTAACTC-3'); atpG-F (5'-ATGGCAGGTGCAA-AAGAGAT-3'), and atpG-R (5'-TTGTACAACAGGC-TTTTGCG-3'); frdB-F (5'-CTTAT-CGTTGGTCTTGCCGT-3'), and frdB-R (5'-TTGG-CACTTTCCACTTTTCC-3'); fucK-F (5'-ACCACTTTCGG-CGTGGATGG-3'), and fucK-R (5'-AAGATTTCCC-AGGTGCCAGA-3'); mdh-F (5'-TCATTGTATGATATTG-CCCC-3'), and mdh-R (5'-ACTT-CTGTACCTGCATTTTG-3'); pgi-F (5'-GGTGAAAAAA-TCAATCGTAC-3'), and pgi-R (5'-ATTGAAAGACCAA-TAGCTGA-3'); recA-F (5'-ATGGCAACTCAAGAAGAAAA-3'), and recA-R (5'-TTACCAAACATCACGCCTAT- 3').

All primers were synthesized by Eurofins, Germany. Primers were supplied lyophilized; first reconstituted to 100 μM (working stock) following the manufacturer's instructions and working concentrations of 10 μM prepared using 1 x TE buffer as diluent.

j) MLST Protocol Set-up

i. *Sample Requirement*

Metagenomic DNA (mDNA) samples of serotyped *H. influenzae* with singleplex Real-time PCR, stored at – 20 ^{O}C (or at – 80 ^{O}C) until required for testing.

ii. *Reagents and Materials*

10 μM *H. influenzae* primer mixes labelled *adk, atpG, frdB, fucK, mdh, pgi*, and *recA* mix, respectively. qPCR Master Mix (SYBR); PCR water (Nuclease free water); ABI One Step Plus Real-time PCR System (Thermofisher, UK); ABI 96 well qPCR plate; P10, P100, and P1000 pipettes and tips; Thermal seal for PCR microtitre plate; Cold rack; Refrigerated centrifuge with plate holder (Heraeus, UK).

iii. *Setting up Reaction*

A worksheet was created according to the number of samples to be tested. The ABI 96 well plate was placed into a plate holder on a cold rack. Note: Each genotypic marker was tested at a time. So, for each sample, there were seven separate reactions. Into each well, 15 μL of qPCR Master mix / Primer was dispensed. Five (5) μL of sample (mDNA), Positive control and Negative control (PCR water) was added into the appropriate well. The plate was sealed with a thermal seal and centrifuged at 1000 rpm for 1 minute in a refrigerated centrifuge (2 – 8 ^{O}C). The microtitre plate was placed into the holder in the ABI One Step Plus Real-time PCR machine. The manufacturer's instruction was followed in setting up the template – *H. influenzae* genotyping. To commence testing, SYBR Chemistry and Standard mode, with Absolute Quantification, were selected. The run was started and saved correctly. The thermal profile comprised of initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 5s, 60°C for 30s, 72°C for 10s, and a final melt cycle of 72°C to 95°C at ramp rate of 0.3°C/s.

iv. *Result Analysis*

After the run, both the amplification curve and cycle threshold (Ct) values were inspected. Ct values of < 35 were positive; Ct values of 35 – 40 were equivocal; Ct values > 40 were negative. For the equivocal ones, the amplification curve and melting curve should be

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checked to decide the result. If it melts at the same specific temperature as the positive cases, it should be considered positive.

v. *Sequenced Results from MLST*

The genome sequences obtained from genotyping (MLST protocol) was uploaded to the publicly available Haemophilus PubMLST.org database (http://pubmlst.org/haemophilus) [Jolley & Maiden, 2014], powered by the Bacterial Isolate Genome Sequence Database (BIGSdb) software [Jolley & Maiden, 2010] for the determination of sequence type (ST), biotype, and global epidemiology status.

vi. *Extracting Typing Information from a Local Genome File*

The MLST scheme selected from a drop-down box of the PubMLST.org database for Haemophilus, was selected, and the analysis run started by clicking the submit button. Individual allelic matches identified along with the sequence type (ST) if the combination of alleles has been previously defined [Jolley et al., 2018]. Typing information can be readily extracted from whole genome sequence assemblies using the sequence query pages. Genome assembly contids pasted into the sequence query form of the database, and the required scheme or locus was selected. Any locus exact matches are displayed, and, if this corresponds to a defined combination of alleles, the profile definition (Sequence type (ST) / clonal complex (cc) for MLST) was displayed [Jolley et al., 2018].

III. Results

Of the twelve genotyped isolates with MLST, six (50 %) had detectable genotypes, while the remaining six (50 %) were undetectable genotype strains. Of the seven genotypes tested, we encountered three: *adk,* two cases (16.7 %); *fucK,* two cases (16.7 %); and *mdh,* two cases (16.7 %) (Table 2). The *H. influenzae* MLST scheme only allows any isolate to be compared with those in the MLST database, and (for encapsulated isolates) it assigns isolates to their phylogenetic lineage, via the internet. Results for *H. influenzae* genotypes sequence profile extracted from PubMLST.org powered by BIGSdb software as shown in Tables 3 to 5. Genotype *adk* sequence profile extracted for serotypes e (*ecsH*) and f (*bexD*) (Table 3); genotype *fucK* sequence profile extracted for serotypes b (*bcsB*) and f (*bexD*) (Table 4); and genotype *mdh* sequence profile extracted for serotypes b (*bcsB*) and e (*ecsH*) (Table 5).

IV. Discussion

Bacterial genotypes obtained from the housekeeping genes are meant to establish if a disease outbreak is short term (local epidemiology) or long term (global epidemiology) [Maiden et al., 1998]. Global epidemiology of pathogenic bacteria requires the direct comparison of isolates obtained in laboratories in different areas of the world in order to track the development of potential outbreaks [Chan et al., 2001]. Of the three genotypes encountered, genotype *adk* was found to be circulating amongst *H. influenzae* serotypes e (*ecsH*) and f (*bexD*) (Table 2). *H. influenzae* genome sequence query on the PubMLST.org for typing by MLST allelic profile through a search by locus combinations showed "No records found". However, under 'genome collection page', which is a subset of records within the isolate database that may contain genomes assemblies; one can access these from the isolate database by filtering on the sequence size in a query [Jolley & Maiden, 2010].

Our query of genotype *adk* by filtering on the sequence size for serotype e (*ecsH*) with identified biotype revealed sequence type (ST) of MLST allele 18, ST 18 from three isolates submitted from Czech Republic, France, and Spain (Table 3). However, serotype f (*bexD*) showed varying sequence types (ST) of MLST (*adk*) allele 22, ST 124, 980, and 1126 (Table 3). All serotype e (*ecsH*) invasive strains belonged to ST 18 [Puig et al., 2014]. However, serotype f (*bexD*) strains were genetically diverse formed by ST 124, ST 980, and ST 1126 as against the report by Puig et al., (2014) which implicated only ST 124 in their study.

Genotype *fucK* was found to be circulating amongst serotypes b (*bcsB*) and f (*bexD*) (Table 4). Our query of genotype *fucK* by filtering on the sequence size for serotype b (*bcsB*) with identified biotype revealed varying sequence types (ST) of MLST (*fucK*) allele 5, ST 6, 54, and 913 (without biotype) from three isolates from Czech Republic, USA, and Nigeria (Table 4). Serotype f (*bexD*) showed varying sequence types (ST) of MLST (*fucK*) allele 11, ST 124, 980, and 1126 from three countries (Czech Republic, Norway, and Spain) (Table 4). Genotype *mdh* was found to be circulating amongst serotypes b (*bcsB*) and e (*ecsH*) (Table 5). Our query of genotype *mdh* by filtering on the sequence size for serotype b (*bcsB*) with identified biotype revealed two sequence types (ST) of MLST (*mdh*) allele 4, ST 6 and 54 from two countries (Czech Republic and USA) (Table 5). Serotype e (*ecsH*) with MLST (*mdh*) allele 10, showed a single Sequence Type, ST 18 from three countries (Czech Republic, France, and Spain) (Table 5).

Therefore, using the principle of inference, the strain designations of our isolates from the global perspective are: (1) *H. influenzae* serotype e (*ecsH*), biotype I, allele 18, ST 18, genotype *adk*; (2) *H. influenzae* serotype f (*bexD*), biotype I, allele 22, ST 124, 980, and 1126, genotype *adk*; (3) *H. influenzae* serotype b (*bcsB*), biotype I, IV, allele 5, 41, ST 6, 54, and 913, genotype *fucK*; (4) *H. influenzae* serotype f (*bexD*), biotype I, allele 11, ST 124, 980, and 1126, genotype *fucK*; (5) *H. influenzae* serotype b (*bcsB*), biotype I, IV, allele 4, ST 6, and 54, genotype *mdh*; (6) *H. influenzae*

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serotype e (*ecsH*), biotype I, allele 10, ST 18, genotype *mdh*.

The identification of *H. influenzae* 'genotype adk' amongst serotypes e (*ecs*H) and f (*bexD*), 'genotype *fuc*K' amongst serotypes b (bcsB) and f (*bexD*), and 'genotype *mdh*' amongst serotypes b (*bcsB*) and e (*ecsH*) indicates an established genetic similarity (or relatedness) between the respective serotypes. Such genetic similarities amongst *Neisseria meningitidis* serogroups has been reported (Lamelas et al., 2017; Peletiri et al., 2022).

V. Conclusion

The MLST results provided an overview of circulating bacterial genotypes of *H. influenzae* and its' genetic diversity status. This information is of great importance for public health strategies such as vaccination. From the global perspective analysis, the strains responsible for infection in Nigeria had global linkage. This could be as a result of travels and movement of persons from one geographical area to another as earlier reported (McGahey, 1905). Finally, as clarified by Puig et al., (2014), all the isolates of *H. influenzae* from CSF with clinical symptoms in the patient, are invasive strains; we document the three genotypes encountered in this research (*H. influenzae* genotypes *adk, fucK,* and *mdh*) as invasive strains reported for the first time in parts of Northern Nigeria. Therefore, for a preventive vaccination programme against cerebrospinal meningitis caused by *H. influenzae* to be successful in Nigeria, these identified genotype strains should be included in the composition of the vaccines for administration.

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Table 1: H. influenzae MLST scheme, including gene locus, amplicon length, and trimmed length of sequence used for allelic determination on http://www.mlst.net platform (CDC, 2011, Chap. 12).

Housekeeping genes	Gene locus	Trimmed length
Adenylate kinase	*adk*	477
ATP synthase F1 subunit gamma	*atpG*	447
Fumarate reductase iron-sulfur protein	*frdB*	489
Fuculokinase	*fucK*	345
Malate dehydrogenase	*mdh*	405
Glucose-6-phosphate isomerase	*pgi*	468
RecA protein	*recA*	426

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Table 2: Prevalence of circulating *H. influenzae* genotypes amongst the various identified serotypes in parts of Northern Nigeria

Gene locus	Allele	Number Encountered	%	Circulating amongst serotypes	No. of serotypes
adk	477	2	16.7	e (*ecsH*) 1; f (*bexD*) 1	2
fucK	345	2	16.7	b (*bcsB*) 1; f (*bexD*) 1	2
mdh	405	2	16.7	b (*bcsB*) 1; e (*ecsH*) 1	2
Undetectable genotype strains		6	50.0		
Total		12	100.0		

Gene locus - Housekeeping genes
adk - Adenylate kinase
fucK - Fuculokinase
mdh - Malate dehydrogenase

Table 3: *H. influenza*e genotype *adk* sequence profile extracted for serotypese (*ecsH*) and f (*bexD*) from PubMLST.org powered by BIGSdb for Sequence Type (ST), biotype, and global epidemiology status

Id	Isolate	Country	Year	Serotype	Biotype	MLST (*adk*) Allele	ST
Serotype e(*ecsH*)							
1217	031/07	Czech Rep.	2007	e	I	18	18
1980	52576	Spain	2008	e	I	18	18
3311	LNP28998	France	2017	e	I	18	18
Serotype f(*bexD*)							
1218	032/07	Czech Rep.	2007	f	I	22	124
1775	702/95	Norway	2011	f	I	22	980
1973	51118	Spain	2005	f	I	22	
9873	30/18	Czech Rep.	2018	f	I	22	124

Table 4: *H. influenza*e genotype *fucK* sequence profile extracted for serotypesb (*bcsB*) and f (*bexD*) from PubMLST.org powered by BIGSdb for Sequence Type (ST), biotype, and global epidemiology status

Id	Isolate	Country	Year	Serotype	Biotype	MLST (*adk*) Allele	ST
Serotype b(*bcsB*)							
105	7854	USA	1990	b	IV	5	54
149	Hi.28/01	Czech Rep.	2001	b	I	5	6
1673	P10095	Nigeria	2010	b	Nil	41	913
Serotype f(*bexD*)							
1218	032/07	Czech Rep.	2007	f	I	11	124
1775	702/95	Norway	2011	f	I	11	980
1973	51118	Spain	2005	f	I	11	1126

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Table 5: *H. influenzae* genotype *mdh* sequence profile extracted for serotypesb (*bcsB*) and e (*ecsH*) from PubMLST.org powered by BIGSdb for Sequence Type (ST), biotype, and global epidemiology status

Id	Isolate	Country	Year	Serotype	Biotype	MLST *(mdh)* Allele	ST
Serotype b(*bcsB*)							
106	7863	USA	1990	b	IV	4	54
149	Hi.28/01	Czech Rep.	2001	b	I	4	6
Serotype e(*ecsH*)							
1217	031/07	Czech Rep.	2007	e	I	10	18
1980	52576	Spain	2008	e	I	10	18
3311	LNP28998	France	2017	e	I	10	18

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Mecanismos De Expressão De Resistência Aos Antibióticos E Saúde Pública

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Abstract- With the advance of medicine and the increase in the use of antimicrobials, microbial resistance has become a serious problem in public health. According to the World Health Organization, resistance to antibiotics is defined when it no longer has an effect. The increasingly frequent insertion of antimicrobials favors resistance, where they put selective pressure on microorganisms, making them resistant to various drugs. For a bacterium to become resistant, several factors are necessary, among them, the indiscriminate and prolonged use of antimicrobials and the intrinsic and acquired resistance. In this context, the objective of the work was to explore the mechanisms of action of antimicrobials, resistance and their importance in public health. Pubmed, Google academic and Scielo databases were used for this research.

Keywords: intrinsic resistance, bacteria, antimicrobials, acquired resistance.

GJMR-C Classification: DDC Code: 362.1 LCC Code: RA241



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Mecanismos De Expressão De Resistência Aos Antibióticos E Saúde Pública

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Resumo- Com o avanço da medicina e o aumento do uso de antimicrobianos, a resistência microbiana vem se tornando um problema sério na saúde pública. Segundo a Organização Mundial da Saúde define-se resistência ao antibiótico quando o mesmo não produz mais efeito. A inserção cada vez mais frequente de antimicrobianos favorece a resistência, onde provocam uma pressão seletiva sobre os microrganismos, tornando-os resistentes a diversas drogas. Para que uma bactéria se torne resistente, são necessários vários fatores, entre eles, o uso indiscriminado e prolongado de antimicrobianos e as resistências intrínsecas e adquiridas. Nesse contexto, o objetivo do trabalho foi explorar os mecanismos de ação dos antimicrobianos, de resistência e a sua importância na saúde pública. Foram utilizadas para a presente pesquisa, as bases de dados Pubmed, Google acadêmico e Scielo. Neste sentido, conclui-se que é de suma importância a atualização de protocolos que contenham os mecanismos de resistência bacteriana a fim de minimizar o uso indiscriminado de antimicrobianos, assim como capacitar os profissionais da saúde para este problema na saúde pública.

Palavras-Chaves: resistência intrínseca, bactérias, antimicrobianos, resistência adquirida.

Abstract- With the advance of medicine and the increase in the use of antimicrobials, microbial resistance has become a serious problem in public health. According to the World Health Organization, resistance to antibiotics is defined when it no longer has an effect. The increasingly frequent insertion of antimicrobials favors resistance, where they put selective pressure on microorganisms, making them resistant to various drugs. For a bacterium to become resistant, several factors are necessary, among them, the indiscriminate and prolonged use of antimicrobials and the intrinsic and acquired resistance. In this context, the objective of the work was to explore the mechanisms of action of antimicrobials, resistance and their importance in public health. Pubmed, Google academic and Scielo databases were used for this research. In this sense, it is concluded that it is extremely important to update protocols that contain the mechanisms of bacterial resistance in order to minimize the indiscriminate use of antimicrobials, as well as to train health professionals for this problem in public health.

Keywords: intrinsic resistance, bacteria, antimicrobials, acquired resistance.

I. Introdução

A resistência bacteriana aos antibióticos é uma das maiores ameaças para a saúde pública global do século 21, sendo necessário grande esforço para contê-la. Haja vista que as infecções causadas por esses patógenos são geralmente mais difíceis de tratar, podem recidivar e causar mortalidade significativa (WHO, 2014).

A resistência da bactéria aos antimicrobianos pode ser definida como a capacidade de uma cepa bacteriana resistir à ação de certo antibiótico, que pode ser mediada pela presença de mecanismos de resistência molecular como as modificações no sítio de ação, a hidrólise enzimática ou transtornos de permeabilidade. Uma vez introduzido o antibiótico, variantes patogênicas com algum mecanismo intrínseco ou adquirido são selecionadas e resistem (PENESYAN, GILLINGS, PAULSEN, 2015).

Os antimicrobianos são fármacos de diversas origens, podendo ser animal, sintética ou semissintética, com ação de destruir (microbicidas) ou inibir o crescimento (microbiostáticos) dos microrganismos. A descoberta desses fármacos melhorou o prognóstico de muitas doenças, antes não combatidas, mudando o curso natural na prática clínica (VIEIRA; VIEIRA, 2017).

Antes do descobrimento dos primeiros antimicrobianos, o controle das infecções no âmbito hospitalar baseava-se na limpeza periódica dos leitos, no isolamento dos pacientes infectados, no tratamento individualizado dos pacientes e na diminuição do fluxo de pessoas que entravam em contato com o doente. Por meio dessas medidas era possível controlar a

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disseminação das bactérias, porém não tratava os pacientes infectados, levando muitas vezes o paciente a óbito (ROCHA et al., 2011).

A descoberta de antibióticos gerou uma mudança significativa no tratamento clínico dos pacientes e na expectativa de vida das pessoas. É possível dividir a história da saúde no mundo em momento anterior e posterior aos antibióticos (SHERPA, REESE, ALIABADI, 2015). Contudo o uso generalizado e indiscriminado dos antibióticos, em humanos, agricultura, pecuária e indústria, levaram a resistência antimicrobiana (Harbarth et al. 2015). Essa resistência acarreta na ineficácia do tratamento nas infecções hospitalares e aumenta os índices de morbidade e mortalidade hospitalares (NEVES; COLET, 2015).

A etiologia da resistência bacteriana é complexa e envolve uma série de fatores, todavia sabe-se que para o controle da resistência são necessárias duas etapas fundamentais; a eliminação cruzada do agente e o desenvolvimento de uma política para promover o uso racional do antimicrobiano (PATERSON, 2006). Baseado no contexto supracitado esse artigo tem como objetivo realizar uma revisão científica sobre os mecanismos de resistência microbiana e o impacto desta na saúde pública.

II. Material e Métodos

Este é um estudo descritivo de revisão da literatura com base nas etapas de identificação do tema e desenvolvimento da questão norteadora; estabelecimento de inclusão e critérios de exclusão; análise e interpretação dos dados e resultados; apresentação da revisão. A busca literária ocorreu a partir de artigos indexados entre 2005 e 2020 em bibliotecas virtuais internacionais, Pubmed (US National Library of Medicine National Institute of Heath), Scielo (Scientific Eletronic Library Online) e Google Acadêmico. Foram utilizados como descritores: resistência antimicrobiana, resistência bacteriana, mecanismos de resistência microbiana, antibióticos e bactérias.

Como critério de inclusão, foram analisados os artigos com base na qualidade de descrição de hipótese/objetivos; prioridade da descrição do desfecho a ser estudado; caracterização da amostra incluída; peculiaridade da descrição e discussão dos assuntos de interesse para o desenvolvimento deste estudo. Por sua vez, estudos repetidos, estudos que não abordam o tema proposto, estudos incompletos, duplicatas, monografias, e publicações de eventos foram excluídas.

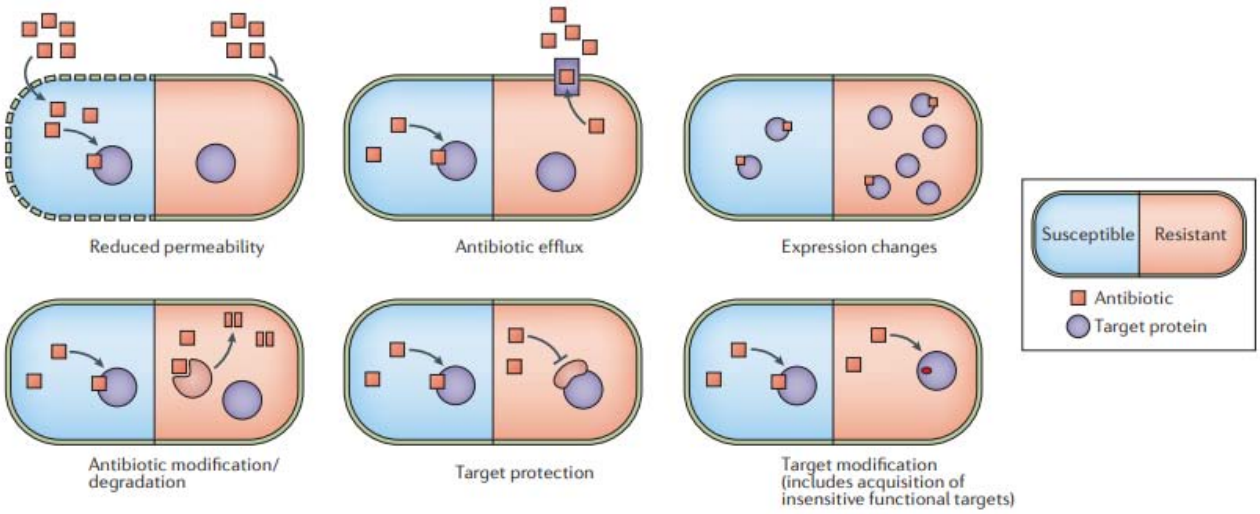
III. Desenvolvimento

a) Mecanismos De Resistência Bacteriana

Os mecanismos de resistência bacteriana se dão muitas vezes por expressão de genes individuais ou em conjuntos, que determinam o funcionamento de resistência, maquinarias bioquímicas ou estruturas que promovem a falha no mecanismo de ação do antibiótico (MOTA et al., 2010).

As bactérias podem apresentar resistência intrínseca ou inerente e resistência adquirida. A resistência intrínseca está ligada ao gênero e espécie das bactérias, já a resistência adquirida acontece através de mutações do próprio gene ou pela aquisição dos genes de resistências de outras bactérias (conjugação, por ex.), através de um bacteriófago, ou ainda, através de um ambiente (HOSPITAL DAS CLÍNICAS UNICAMP, 2019).

Os principais mecanismos de resistência são: produção de enzimas que degradam ou modificam o antimicrobiano, redução da permeabilidade da membrana, sistema de efluxo hiperexpresso, alteração do sítio alvo do antibiótico, bloqueio ou proteção do sítio alvo do antibiótico (SILVA, 2008).



Fonte: Modificado de Boolchandani et al. (2019)

Figure 1: Principais mecanismos de resistência bacteriana.

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b) Produção De Enzimas Que Degradam Ou Modificam O Antibiótico

As bactérias podem conter genes que codificam a produção de enzimas com propriedades de modificar ou inativar irreversivelmente antibióticos, como as β-lactamases, as enzimas modificadoras de aminoglicosídeos ou as cloranfenicol acetiltransferases (SANTAJIT, INDRAWATTANA, 2016).

Existem dezenas de tipos de β-lactamases, variando de substrato e microrganismo produtor. A classificação comumente utilizada é a de Ambler que divide as β-lactamases em quatro classes: A, B, C e D (SANTAJIT, INDRAWATTANA, 2016).

Tabela 1: Exemplos das quatro classes, resistência e possíveis opções terapêuticas

Enzima	Exemplo	Resistência	Possíveis opções terapêuticas
CLASSE A			
β-lactamases de espectro estreito (Penicilimases)	*Staphylococcus*	Penicilinas naturais e aminopenilinas	Oxacilina Amoxilina-clavulanato
β-lactamases de amplo espectro	Enterobactérias	Penicilinas, Cefalosporinas e Aztreonam	Carbapenêmico
Carbapenemases	*Klebsiella pneumoniae Esccherichia coli*	Todos os β-lactâmicos	Ceftazidima-Avibactam
CLASSE B			
Metalo-β-lactamases	Enterobactérias *Pseudomonas aerugionisa*	Todos os β-lactâmicos (exceção Aztreonam) e inibidores da β-lactamase	Aztreonam
CLASSE C			
Cefalosporinases	Enterobactérias	Penicilinas, Cefalosporinas até terceira geração	Carbapenêmico
CLASSE D			
Oxacilinases	Enterobactérias *Acinetobacter baumannii*	Penicilinas e Carbapenêmicos	Ceftazidima-Avibactam

Fonte: Adaptado Poton (2019)

As penicilimases são produzidas por uma variedade de bactérias, sendo *Staphylococcus aureus*, *Haemophilus influenza* e algumas bactérias gram negativas, exemplos de microrganismos produtores. Geralmente esse tipo de resistência atua sobre penicilinas lábeis, como amoxicilinas e penicilinas. A oxacilina por ser uma penicilina estável, pois não sofre ação dessa enzima (BAPTISTA et al., 2013).

As cefalosporinases, cefamicinases, beta-lactâmicos de amplo espectro e carbapenemases são produzidos por gram negativos (*Escherichia coli*, *Klebsiella* spp, entre outras) ou bactérias não fermentadoras (*Pseudomonas aeruginosa*, *Acinetobacter*) (BLAIR; WEBBER, 2015). As beta-lactamases de amplo espectro (ESBL) são enzimas capazes de degradar todas as penicilinas, cefalosporinas e monobactâmicos, sendo sensíveis a cefamicinas e aos carbapenêmicos (BLAIR; WEBBER, 2015).

As carbapenemases são enzimas capazes de degradar os antibióticos carbapenêmicos. São enzimas codificadas por genes plasmodiais ou cromossômicos. Elas são divididas em dois grupos: Metalo-carbapenemases ou serina carbapenemases. O metalo-carbapenemases possui a capacidade de degradar todos os beta-lactâmicos, exceto os monobactâmicos (aztreonam) (BAPTISTA et al., 2013).

c) Enzimas Que Alteram O Antibiótico

Existem enzimas que transferem alguns grupos químicos para as moléculas dos antimicrobianos, mudando sua conformação e inativando-as. Atuam em aminoglicosídeos, macrolídeos e fenicóis. As enzimas modificadoras de aminoglicosídeos são as principais

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formas de resistência desse grupo, pois alteram a estrutura química, impedindo a ligação com as subunidades dos ribossomos (DŽIDIĆ; ŠUŠKOVIĆ, 2008).

d) Redução Da Permeabilidade Externa

Para que os antimicrobianos tenham efeito, eles necessitam atravessar a parede celular e chegarem até o meio intracelular. A resistência ocorre pela mudança na conformação da parede celular e pela alteração nas estruturas das porinas ou ausência delas, resultando em uma permeabilidade seletiva ou até mesmo na impermeabilidade as drogas. Esse tipo de resistência diminui a sensibilidade dos antimicrobianos a cefoxitina e cefepime, e costuma afetar a sensibilidade dos carbapenêmicos (DA COSTA; JUNIOR, 2017).

Devido à conformidade da parede celular das gram negativas serem menos permeáveis do que as gram positivas, esse tipo de resistência se dá exclusivamente nas gram negativas (BLAIR; WEBBER, 2015).

e) Sistema De Efluxo Hiperexpresso

Sistema de efluxo são mecanismos naturais das bactérias, codificadas por genes cromossômicos, com a função de excretar substâncias tóxicas resultantes dos metabolismos. A resistência ocorre quando possui um aumento da atividade desse mecanismo ou por aumento desses sítios. Macrolídeos, fluoroquinolonas e tetraciclinas são exemplos antimicrobianos que sofrem ação desse mecanismo de resistência (BAPTISTA et al., 2013).

f) Alteração Do Sítio Alvo Do Antibiótico

Antimicrobianos ligam-se a um ou mais sítios na célula bacteriana, alterando o sitio de ligação, a afinidade da ligação, diminuindo ou ocorrendo a perda da eficiência da ligação. Geralmente essa mutação ocorre no gene da própria bactéria, porém a proteína alterada não perde sua função (DA COSTA; JUNIOR, 2017).

Um exemplo desse mecanismo de resistência são as mutações na região de resistência as quinolonas. Ocorrem alterações nos genes gyrA e/ou parC alteram as enzimas topoisomerase IV e/ou DNA gyrase que atuam na duplicação do DNA. (DA COSTA; JUNIOR, 2017).

Outro exemplo são as proteínas ligadoras de penicilinas (PBP, Penicillin Binding Proteins). Essa alteração ocorre em *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus mitis* e *Neisseria gonorrhoeae* provocando resistência a diversos beta lactâmicos (DŽIDIĆ; ŠUŠKOVIĆ, 2008).

A SCCmec (*Staphylococcal Cassette Chromossome* mec), codifica um gene PBP que está presente no S. *aureus*, promovendo a resistência a meticilina. Quando esse gene for detectado, indica que as bactérias são resistentes a todos os outros beta-lactâmicos, devendo ser substituído por glicopeptídeos (vancomicina ou teicoplanina) (DA COSTA; JUNIOR, 2017).

A última alteração desse grupo ocorre no terminal, D-alanina-D-alanina. Geralmente ocorre em *Enterococcus* e são responsáveis pela produção da glicoproteína da parede celular e pelo sitio alvo de alguns antimicrobianos, como a vancomicina. Ocorre devido a incorporação de um elemento genético Transposon TN1546, que carrega os genes vanA, vanB, vanC que codificam um terminal D-Alanina-D-lactato, causando resistência a vancomicina (BLAIR; WEBBER, 2015).

g) Proteção Ou Bloqueio Do Sitio Alvo

Nesse mecanismo de resistência, os microrganismos produzem enzimas ou estruturas celulares que impedem a ligação do antimicrobiano ao sítio alvo. A enzima Qnr, mediada por genes adquiridos (PMQR - Plasmid Mediated Quinolone Resistence) que liga-se ao DNA tropoisomerase 2 dos microrganismos e impede a ação das quinolonas (BAPTISTA et al., 2013).

Outro exemplo, também ocorre em *Staphylococcus aureus,* onde ocorre um aumento na espessura da parede celular, mediada pela expressão de genes, que impede ação do glicopeptídeos ao sitio alvo. Esse bloqueio pode ser parcial (se expressado os genes VISA/GISA) ou total (se expressado os VRSA/GRSA) (DA COSTA; JUNIOR, 2017).

h) Formação De Biofilme

A formação de um biofilme é considerada uma nova forma de resistência, de acordo com o Freire et al. (2018). Podem ocorrer em vários lugares, como por exemplo, placas dentárias, cateter médicas e feridas traumáticas. Nesses lugares, os microrganismos são encontrados protegidos por biofilmes, da entrada de diversos agentes microbianos (DA COSTA; JUNIOR, 2017).

A formação do biofilme acontece por duas etapas: a primeira ocorre adesão das células a uma superfície e na segunda ocorre a formação de micro colônias que se acumulam em multicamadas, iniciando a síntese da matriz extracelular que formará o biofilme. Ocorre intensa comunicação entre as células microbianas, que coordenadas pelos genes, formam um exopolissacarídeos ativados, onde irão depositar agregados de células que irão constituir o biofilme maduro (SOUSA; BOTELHO; OLIVEIRA, 2011).

i) Ação Dos Antimicrobianos

Antibióticos são fármacos utilizados no tratamento de doenças infecciosas, dividido em classes que diferem uma da outra pelo seu mecanismo de ação, propriedades químicas, físicas, farmacológicas (BAPTISTA et al., 2013).

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Para que o antimicrobiano seja efetivo, ou seja, com resultados terapêuticos ideais, depende de alguns requisitos como a sua concentração, não podendo ter inativação ou mudança de conformação, além de ter receptores nas bactérias. O antimicrobiano precisa ter ação bactericida sobre a bactéria específica, alvo seletivo, que não afete a microbiota normal, alto efeito terapêutico, poucas reações adversas, e principalmente, que não induza resistência microbiana (intrínseca) (DA COSTA; JUNIOR, 2017).

Os antimicrobianos podem ser do tipo natural (obtidos a partir de microrganismos vivos), semissintéticos (são de origem natural, porém sofre processos de síntese em laboratório) e sintéticos (produzidos exclusivamente em laboratório). Podem ser bacteriostáticos, ou seja, impede a crescimento bacteriano ou bactericida, possuem a ação de destruir as bactérias. Por último, a classificação de acordo com ação farmacodinâmica: Inibição da síntese da parece celular, inibição da síntese proteica, inibição de ácidos nucléicos, desorganização da membrana celular, interferência no metabolismo celular (GUIMARÃES; MOMESSO; PUPO, 2010).

j) Inibição Da Parede Celular

Os antimicrobianos desta classe atuam inibindo a síntese do peptídeoglicano, como por exemplo, os antimicrobianos, penicilina e as cefalosporinas. Eles inibem a síntese de enzimas necessárias para formação da parede de peptídeoglicano. Os glicopeptídeos, exemplo é a vancomicina, tem como ação a ligação a extremidade D-ala-D-ala da cadeia do peptídeoglicano, impedindo a ligação da N-glutilglucasamina e o ácido N-acetilmurâmico que forma o peptídeoglicano da parede celular (DŽIDIĆ; ŠUŠKOVIĆ, 2008).

k) Inibição Da Síntese Protéica

Os ribossomos das bactérias são organelas compostas por 2 subunidades, 30S e 50S, onde ocorre a síntese de proteínas. São pertencentes a essa classe de fármacos: aminoglicosídeos, tetraciclinas, clorafenicol, macrolídeos, licosamida, oxazolinidonas. Esses fármacos atuam sobre uma das duas subunidades formadoras dos ribossomos, impedindo a síntese de enzimas necessárias para o metabolismo e o crescimento bacteriano (DA COSTA; JUNIOR, 2017).

l) Inibição Da Síntese De Ácido Nucléicos

Essa classe de fármacos possui ação de inibir a enzima girase e tropoisomerase IV que atuam na replicação do DNA. São exemplos dessa classe as quinolonas: ciprofloxacino, norfloxacino, oxafloxacino. A rifampicina, pertencente a esse grupo, possui o mecanismo diferente, atuando na inibição do RNA polimerase, responsável pelo processo de transcrição e impedindo a síntese de RNA mensageiro (DŽIDIĆ; ŠUŠKOVIĆ, 2008).

m) Desorganização Da Membrana Celular

As polimixinas são representantes dessa classe, que são moléculas anfipáticas tensoativas que interagem com as moléculas de polissacarídeos da membrana externa, sequestrando cálcio e magnésio, desestabilizando a membrana celular, provocando a alteração da permeabilidade e o extravasamento do conteúdo celular (DŽIDIĆ; ŠUŠKOVIĆ, 2008).

n) Interferência No Metabolismo Celular

Representantes dessa classe atuam em alguma fase da formação do folato, cofator necessário para síntese de DNA e RNA. Sulfonamidas e trimetoprima possuem esse mecanismo de ação, onde atuam em associação, sendo que cada um atua em uma etapa do metabolismo. As Sulfonamidas atuam bloqueando a enzima diidropteroato sintetase, presentes apenas nas bactérias. O trimetoprima inibe a diidrofolato redutase. Com esse bloqueio não se forma a metilenotetrahidrofolato, um importante cofator na formação das bases pirimidínicas dos ácidos nucléicos (WALSH et al., 2003).

o) Resistência Microbiana Na Saúde Pública

Com a preocupação da resistência microbiana, algumas intervenções devem ser tomadas para controle e não disseminação: educação continuada dos profissionais, acompanhamento de paciente de risco (com culturas de vigilância), isolamento de pacientes colonizados ou contaminados, uso de Equipamentos de Proteção Individual (EPI´s), lavagem das mãos, uso racional de antibióticos, entre outros (BLAIR; WEBBER, 2015).

Uma infecção começa quando um agente invade o hospedeiro, compromete o sistema imunológico de defesa e coloniza os tecidos, provocando lesões nos tecidos e alterando as funções fisiológicas, causando a doença (SAKER et al., 2004).

Nesse contexto, o desenvolvimento de ações educativas, de planejamento e organização para prevenção e contenção da doença é de suma importância. Ambientes hospitalares, ambulatoriais e domésticos são onde as ações devem ser realizadas, sendo o patógeno resistente ou não (MEIRELES, 2008).

O uso indiscriminado de antimicrobianos é o principal fator de resistência microbiana, assim como o uso de antimicrobianos sem exame de cultura e teste de sensibilidade. Esses dois fatores são os principais no desencadeamento de patógenos de multirresistentes em ambientes hospitalares, onde os fármacos exercem pressão seletiva sob as cepas resistentes. Tem que se levar em conta, o fator socioeconômico, onde o paciente muitas vezes não possui acesso aos antimicrobianos corretos e se recusam procurar um atendimento médico, utilizando outros (DA COSTA; JUNIOR, 2017).

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IV. Conclusão

Os antimicrobianos representam um grande avanço na medicina. A partir deles que procedimentos invasivos como cirurgias, transplantes, terapias oncológicas foram possíveis de serem realizados.

O uso indiscriminado dos antimicrobianos traz uma série de problemas para saúde pública, como a resistência microbiana e a disseminação de cepas de bactérias resistentes dentro dos ambientes hospitalares e na comunidade.

Diante desse contexto, são necessárias formulações de materiais educativos para a população e campanhas que orientem ao risco do uso indiscriminado dos antibióticos, assim como orientar profissionais da saúde para o uso racional, evitando a prevalência de muitas endemias. Sendo assim, mas estudos e maneiras de amenizarem as resistências microbianas são necessários para que aumente a qualidade de vida dos pacientes doentes.

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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

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Format Structure

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All manuscripts submitted to Global Journals should include:

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The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

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Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

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Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

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Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

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Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

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7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

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11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

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22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

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- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

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An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

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Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

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This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

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Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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