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Abstract

The antibacterial potential of pap slurry liquor on four diarrheal associated organisms (Escherichia coli, Staphylococcus aureus, Salmonella tyhpi, and Shigella dysenteriae) was screened for. The pap slurry liquor was obtained in the laboratory from a pap (made from yellow Zeamays grains) which was allowed to ferment at ambient temperature of 28±1oC for 72 hours at relative humidity 75±5

Index terms— pap slurry, antibacterial, diarrheal, organisms.

1 Introduction

ap slurry is a fermentation product from cereals found predominantly in Southern Nigeria and is usually the first native food given directly or supplemented with other food sources to babies at weaning. Ajanaku and Oluwole (2013) reported the use of pap slurry as a weaning food in western Nigeria to supplement breastfeed. It has also been shown that pap liquor has both anti-bacterial (Adebolu et al., 2007) and antifungal properties (Ogunbanwo et al., 2003).It is usually prepared from fermented maize, sorghum or millet in West Africa (Akingbala et al., 2012). It is a popular breakfast cereal and infant weaning food in Nigeria (Akingbala et al., 2012).

In most rural communities, where they do not have access to orthodox medicine, all kinds of plants or raw materials are exploited to take care of the different health challenges they encounter. For example in some communities in the Southwest Nigeria, uncooked papslurry, which is a Nigerian fermented food made from cereal grains such as maize (Zea mays) is used traditionally for the relieve of stomach discomfort and diarrhoea by the rural people. Olukoya et al., (2012) when carrying out research observed that pap slurry has antibacterial activity against common diarrhoeagenic bacteria and that the presence of Lactobacilli in the slurry was responsible for its effect. Adebolu, (2007)in her own contribution however reported that not only the slurry but the liquor also plays a significant antibacterial activity against diarrhoeagenic bacteria and that the growth inhibitory activity was more potent than the slurry on most of the organisms tested.

Moreover, Adebolu, (2007) has observed that the fermentation duration of pap slurry plays a significant role in the growth inhibitory activity of the liquor on susceptible organisms. Furthermore, Adebolu and Adaramola (2012) observed that the mode of fermentation, whether continuous or discontinuous at every 24 h at 30 ± 2 o C, plays a significant role in the inhibition. Although, a lot of work has been done on the antibacterial activity of the slurry of pap, more is still desired so that all necessary scientific intricacies will be taken care of for its usage to be maximally exploited. This present work therefore will help to determine the factors present in the slurry of fermented maize responsible for its antibacterial activity on the selected diarrhea causing bacteria and which one of the factors is the most effective.

Bacteria are known to cause gastrointestinal infections globally. Treatment of infections caused by these organisms is difficult because most bacteria causing infections have developed resistance to most of the conventional antibiotics, and therefore there is the need to search for alternative therapy to treat infections caused by these organisms, hence the pap slurry.

2 II.

3 Materials and Methods

Materials include: conical flask, beaker, petri dishes, test tubes, distilled water, methanol, Mueller Hiltonagar (MHA), wire loop, aluminum foil, cotton wool, spatula, autoclave, incubator, weighing balance, McCartney bottles, physiological saline, Bunsen burner and pipette.

4 a) Sterilization of glass wares

All glass wares were thoroughly washed with detergent and rinsed with distilled water, dried in hot-air oven and then sterilized at 60 o C for 1-2 hours. The work bench surface was disinfected before and after carrying out any experiment to avoid contamination and to ensure aseptic working condition.

5 b) Preparation of culture media

All culture media were prepared according to manufacturer's specification. After proper dissolution the, the media was sterilized in an autoclave at 121 o C for 15minutes. The sterile medium was allowed to cool to about 45 o C before dispensing into sterile petri dishes.

6 c) Fermentation of pap to obtain its liquor

The Yellow maize (Zea mays, grains) was purchased at a local market in Anyigba Kogi State, Nigeria. Using a modified method of Odunfa and Adeleye (1985), the maize grains were carefully sorted by hand picking damaged and infested grains and pebbles, after which they were washed insterile distilled water to remove dirt. Certain grams (2 kg) of the clean maize grains were steeped in two liters sterile water which was sufficient to cover the grains to avoid contamination. The steeped maize was left at room temperature (28 $\pm$ 1°C) for 72 hrs, after which it was drained and washed with sterile water three times and then wet milled using a clean grinding machine. Sieving of the resulting paste was done using a clean muslin cloth and the filtrate was collected in a clean plastic container and left for 72 hrs at room temperature (28 $\pm$2°C) for spontaneous fermentation to take place. At the end of the fermentation, the liquor of the fermented maize slurry (i.e the supernatant solution) which is locally called 'Omi-ogi' was decanted into a sterile container for analysis.

7 d) Test organisms

The Stock cultures of the clinical isolates used for this study were obtained from Medical Laboratory, Kogi State, University Teaching Hospital, Anyigba and confirmatory tests were carried out at the Microbiology Laboratory of the same university.

8 e) Confirmatory test on isolates i. Indole test

The colonies were added into peptone water and incubated for 24 hours at 37 o C after which 3 drops of kova'sindole reagent was added and shaken gently. A red colour development within a minute indicated a positive test.

9 ii. Motility test

The motility test was carried out using a glass slide and a cover slip. Vaseline gel was used to form a ring on the slide and a loopful of the fluid culture (growing on peptone water) was transferred on the cover slip. The slide was inverted over the cover slip so that it adheres to the Vaseline gel, the slide was turned quickly so that the drop does not touch the slip or Vaseline gel. It was observed under a light microscope for characteristics movement.

10 iii. Citrate utilization test

A broth culture of the test organism was incubated in 3ml of koser's citrate medium at 37°c for 3 days. It was checked daily for growth. Presence of blue colouration and turbidity indicated a positive test.

11 iv. Urease test

A tube of sterile motility-indole-urea (MIU) medium was inoculated with the colony of test organisms. An in dole paper strip was placed in the neck of the MIU tube above the medium and it was incubated at 37C overnight. Production of urease was indicated by a red-pink colour in the medium.

12 f) Gram staining

A heat fixed of each Organism was made after which crystal violet was applied for 1-2 minutes and washed with water. The slide was flooded with Gram's iodine for 1 minute and washed with water. The slides were held in slanting positions while absolute alcohol solution was flooded over it until the blue colouration leaves the smear; it was flushed with water and drained. The slide was then counterstained with safranin solution for 30 seconds and washed under slow running water. It was blotted and observed under a light microscope. Gram negative organisms stained red or pink colouration while Gram positive organisms stained blue.

13 g) Preparation of cell suspension

Using physiological saline, cell suspensions was prepared to give concentrations equivalent to McFaland No7 (2.1$\times$10 9 cells/ml). Then, 0.01ml of organisms was used for further inoculation in further testing.

14 h) Preparation of liquor concentration

A 9 ml of pap slurry liquor concentration was diluted in 1ml of sterile distilled water to make a concentration of 90: 10v/v. Other concentration (80:20, 70:30 and 60:40 v/v) was also made following the same procedure.

15 i) Antibacterial screening of the liquor

The surface of the MHA plate was inoculated with the test organisms. Inoculum was standardized by matching the turbidity with 0.5% McFarland standard and then with a sterile cotton swab stick, the test culture was spread evenly over the plate successively in three directions to obtain an even Inoculum. The plate was allowed to gel for 3-5 min. The filter papers discs (6 mm, with average fluid uptake 18 µl) prepared were impregnated into different concentrations of the pap slurry. Commercially available readymade antibiotic disc (cephalexin) was placed on the surface as control and filter paper disc (6mm) impregnated in sterile distilled water was used as the negative control. The plate was incubated overnight at 37°C and the zone of inhibition was measured.

16 j) Determination of minimum inhibitory concentration (MIC)

Tube dilution method was used in the determination of MIC. The MIC was determined for each of the test organisms at the varying concentrations of the liquor. Each test organism was inoculated into the labeled tube by taking a loopful of the standardized bacterial suspension using a flame sterilized wire loop and was incubated at 37 o C for 24 hours.

The lowest concentration where no turbidity was observed was recorded as the MIC.

17 k) Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined using standard method. The tubes that showed no visible growth from the test tubes used in the determination of MIC, were sub cultured onto freshly prepared Mueller Hinton agar and incubated at 37 o C for 48 hrs. The least concentration at which the organisms did not recover and grow was taken as the MBC.

18 l) Data analysis

All the data obtained were subjected to one way analysis of variance at 0.05 significant levels using the New Duncan's Multiple Range Test.

19 III.

20 Results

21 Key: CN = cephalexin SDW= Sterile distilled water

Each value is the mean of three replicates, mean with the same letter are not significantly different (P>0.05) from each other, using New Duncan's Multiple Range Test.

22 Discussion

Pap slurry liquor used in this study had antibacterial activities against all the test bacteria isolates at varying concentrations. A dose dependent relationship was observed. This was evident by the clear zones of inhibition produced by the liquor on the bacteria growth (Table 1). The reports of Oyetayo and Osho (2004) and Aderiye et al., (2007), showed the antibacterial properties of maize pap slurry liquor in vitro on some organisms. In this study, the highest inhibition was recorded against Gram-negative E.coil which was most susceptible to the pap slurry liquor with the maximum zone of inhibition 15 mm at 100 v/v concentration, while the least inhibition was on Staph. aureus (8 mm) at the same concentration. However, no inhibition zone was recorded for Staph. aureus at lower concentrations used (Table 1).

It is worthy of note that the standard antibiotic cephalexin used as control was more potent on the Gram positive organisms (S. typhi and Staph.aureus) than the Gram negative ones (E.coli and S. dysenteriae). Cephalexin is a ?-lactam antibiotic in the class of the first generation cephalosporin which mode of action is by disrupting the growth of the bacterial cell wall. In contract, the pap slurry liquor is more potent on the Gram negative organisms than the Gram positive ones. This may be due to the paucity of peptidoglycan and that the lipopolysaccharide is very thin and sits within the periplasmic space. Shigellae are intracellular parasites that are often transmitted by fecal-oral route primarily by food that multiply within the villus cells of the colonic epithelium. The ability of the liquor to inhibit Shigellasp in this study is of great interest and this corroborates the use of this pap slurry liquor as alternative therapy in the treatment of dysentery. This noticeable anti-diarrhogenic efficacy of pap slurry liquor could be associated with the antagonistic effects of the organisms present in the fermented liquor. Ijabadeniyi, (2007)who worked on the microorganisms associated with ogi

produced from three variety of maize was able to identify Lactobacillus plantarum, Lactobacillus. fermentum, yeast and Saccharomyces cerevisiae in the fermented slurry. Some of these organisms have been a very good potential source of probiotics.

Minimum inhibitory concentration value recorded (Table 2) was 90:10v/v concentration for E.coli, and Staph.aureus while 80:20 v/v was recorded for Staph. aureus, and S. dysenteriae. The results of the minimum bactericidal concentration recorded (Table 3) were 100v/v for E.coli and S. typhi while 90:10v/v concentration, for Staph.aureus, and S. dysenteriae.

V.

23 Conclusion

The results from this study showed that pap slurry liquor from maize (Zea mays) was potent against the diarrheal associated isolates tested. The findings in this study justified the use of pap slurry liquor in the treatment of diarrhea in folklore medicine and the use could be adopted as well since it is cheaper and good source of potent probiotics. However further investigation should be conducted on the maize crop to ascertain the active antimicrobial compounds and the probable mode of actions.

1

	Mean Zones of inhibition (mm) ± SE			
Concentrations v/v	Test organisms			
	Gram - E.coli	Gram - S.dysenteriae	Gram + Staph.aureus	Gram + S. typhi
100	15.0±0.33 g	12.0±0.33 f	8.0±0.00 d	10.0±1.17 e
90:10	13.0±0.00 f	10.0±1.17 e	6.0±1.67 c	9.0±1.67 e
80:20	7.0±1.16 d	8.0±1.17 d	3.0±0.32 b	7.0±2.89 d
70:30	5.0±0.00 c	4.0±1.67 b	0.0±0.00 a	5.0±0.33 c
60:40	3.0±1.67 b	3.0±0.00 b	0.0±0.00 a	4.0±0.33 b
CN(25µg)	16.0±0.00 g	20.0±0.33 h	23.0±0.33 i	15.0±0.67 g
SDW	0.0±0.00 a	0.0±0.00a	0.0±0.00 a	0.0±0.00 a

Figure 1: Table 1 :

2

Concentration v/v	Test of organisms			
	E.coli	S. dysenteriae	Staph.aureus	S.typhii
100	-	-	-	-
90:10	-	-	-	-
80:20	+	-	+	-
70:30	+	+	+	+
60:40	+	+	+	+

Key:
-= no growth recorded.
+=growth recorded

Figure 2: Table 2 :

3

Test organisms	MBC v/v
E.coli	100
Salmonella thyphii	90:10
Staphylococcus aureus	100
Shigelladysenteriae	90:10

IV.

Figure 3: Table 3 :

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