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Research Article

BIOCHEMICAL IDENTIFICATION AND CULTURAL CHARACTERIZATION OF SOME GRAM- NEGATIVE BACTERIA OBTAINED FROM FECAL/ DIARRHOEAL SAMPLES

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ABSTRACT
The fecal/ diarrheal samples were inoculated on to the media for primary investigation and sub- cultured on to the different media for careful isolation and characterization based on the colony formation. The inoculums of each isolate was subjected to IMVIC Test; indole, methyl red, vorges proskauer and citrate test for the possible detection and characterization of the isolates found in the samples obtained. The test revealed that, the isolates were indole and methyl red positives whereas, they were negatives in the case of vorges proskauer and citrate test as well. The most common isolate was *E. coli* (86.9%), followed by *Salmonella* (76.9%) and lastly *Shigella* with 73.1%.

Keywords: *Isolates, Biochemical, IMVIC and Cultural Characterization*

INTRODUCTION
Many pathogenic bacteria such as *Salmonella* and *Shigella* are transmitted by faeces, contaminated food or water, when such food or water taken in, symptom of diarrhea may develop (Jonas *et al*., 1997). However, the role of normal inhabitant of the gastrointestinal tract in human enteritis has not been clearly understood.
The work of Jonas *et al*., (1997), had shown that indigenous bacterial strains of *Klebsiella* were also additional potential but their importance in acute diarrheal illness by children was only partially understood. *Shigellae* cause diseases ranging from diarrhea to bacillary dysentery, an invasive infection of the human colon affecting humans in developing countries, and are associated with poor hygiene conditions. In developed countries, common-source of outbreaks occurs sporadically and several outbreaks have been reported, that the diarrheal disease is highly contagious due to its low infectious dose (Mukhaerjee *et al*., 1998).
Several members of enterobacteriaceas are responsible for causing several infections (Fabio *et al.,* 2007). *Klebsiella pneumonia* and pseudomonas spp, are emerging as an important cause of neonatal nosocomial infections. *Escherichia coli* causes septicemians diarrhea and can infect the gall bladder, meninges, skin lesions and the lungs especially in debilitates and immunodeficient patients. However, Proteus mirabilis cause wound infections following catheterization or cystoscopy and it is a secondary invader of ulcers, pressure sores, etc. Staphylococcus and Streptococcus cause nosocomial infections, food poisoning upper respiratory infection and other type of infection (Pandey, 2007; Mishra *et al.,* 2011). The objective of this research is to identify and characterized some of the gram negative bacteria from fecal/ Diarrheal samples collected.

MATERIALS AND METHODS
Test Organisms
The clinical isolates were obtained from fecal/diarrheal samples were collected from Murtala Muhammad specialist hospital Kano, Kano state. The organisms include; *E. coli, Shigella* spp, *Salmonella* spp, etc. The isolates were indentified using the schemes of Cheesbrough (2006) and then sub-cultured into Mac conkey agar, Eosine methylene blue and Salmonella – Shigella agar for further confirmation Cheesbrough (2006).

Culturing and Isolation of the Test Organisms

A sterile wire loop has been used to inoculate stool samples on Mac conkey agar (MARK). The culture has then incubated at 370C for 24 hours. The pale colonies show the presence of both *Shigella* and *Salmonella*, while *E. coli* appears pinkish in colour. *Shigella, Salmonella* and *E. coli* are all gram negative bacteria (Cheesbrough, 2000). However, during inoculation, the plates of the media have been dried because of easier growth and identification of the colonies. The wire loop has also flamed and sterilized. The plates were placed invertedly overnight, to prevent falling of condensed water vapour on plate surface (Cheesbrough, 2000).

Gram Staining Technique

Thin smear of about 200mm in diameter was made on grease free slide which was also fixed over a burning flame. A crystal violet solution was applied to cover the smear for 30 seconds and it was then washed with distilled water.

Secondly lugol's iodine was applied to the surface for good 30 seconds. Acetone was used to decolorize the stain and lastly, the safranin solution was covered on the surface for a minute, which has been washed and allowed to dry at room temperature.

Then the stains have been observed under microscope with oil immersion. Consequently, red stains indicate gram- negative bacteria (Cheesbrough, 2000).

Biochemical Identification of Bacterial Isolates

All isolates were subjected to the following biochemical tests: - IMVIC (Indole, methyl red, Voges-Proskauer and Citrate).

Both the methyl red and Voges-Proskauer tests were commonly used in conjunction with the indole and citrate tests, to form a group of tests known as IMVIC which aid in the differentiation of Enterobacteria (Holt, 1994).

Indole Test: This confirmed the *E .coli, Shigellae and Salmonellae* from suspected colonies. A wire loop was used to inoculate over night growth cultures into a test tube of 5ml peptone water. The inoculation was incubated at 370C for 24 hours after which 5 drops of Kovac's indole reagent was added and shaken gently. A positive reaction was indicated by the development of a red color formation on the top layer. *E. coli and Shigella* were indole positive while *Salmonella* was indole negative (Cheesbrough, 2000).

Methyl Red- Voges-Proskauer: Organism was grown in 5ml MR-VP broth and incubated for 48-72 hrs at 350C after incubation, 1ml of the broth was transferred into a test tube and 2-3 drops of methyl red was added. Formation of red color indicates positive methyl red test, a yellow color indicates negative test. To the rest of the broth, 15 drops of 15% alpha – napthal in alcohol was added. 5 drops of 40% KOH was also added followed with shaken gently. The cap of the tube was loosened and development of a red color within 1hr indicates a positive test. No color change indicates negative test (Cheesbrough, 2000).

Citrate Test: The isolates were inoculated into Simmon's citrate agar in a Bijou bottle and incubated for 24- 72hrs. Development of a deep blue colour indicates a positive reaction (Cheesbrough, 2000).

Motility: The isolate was inoculated into the motility medium by making a fine stab with a needle to a depth of 1-2cm long in the tube. It was then incubated at 350c for 24 48hrs. Sharply undefined line of inoculation and cloudiness in the media indicates a positive result (organism is motile). Sharply defined and restricted growth to the line of inoculation indicates a negative result (Cheesbrough, 2000).

RESULTS AND DISCUSSION

Results

Table 1: Shows Biochemical reactions and cultural characterization of the isolated organisms found in fecal/diarrheal samples.

The following organisms were identified under IMVIC, the organisms include: *E.coli, Salmonella and Shigella. E.coli and Salmonella* were indole positive while *Shigella* was the only indole negative among them. All the isolates were methyl red positives and at the same time, were all negatives in Voges proskauer and Citrate tests. Morphologically, *E. coli* appeared pinkish, but *Salmonella and Shigella* appeared white and pale in colour.

Table 1: Shows the Biochemical Reactions and Cultural Characterization of the Isolated Organisms Found in Fecal /Diarrheal Samples

Colony Characteristics	Gram Reaction	Motility Test	Methyle Red	Voges Proskauer	Indole	Citrate	Inference
Pinkish colour on EMB	+	_	+	_	+	_	*E. coli*
Whitish colour on SS	+	_	+	_	_	_	*Shigella*
Pale colour on SS	+	+	+	_	+	_	*Salmonella*
Pinkish colour on Mac	+	_	+	_	+	_	*E.coli*
Whitish colour on Mac	+	_	+	_	_	_	*Shigella*
Pale colour on Mac	+	+	+	_	+	_	*Salmonella*

Key: EMB= Eosine methylene blue agar, SS = Salmonella – Shigella agar, Mac = Mac conkey agar , - = negative and + = positive

Table 2: Revealed the Occurrence of Isolates (*E .coli, Shigella* and *Salmonella*) in Fecal/Diarrheal Samples Collected from Murtala Muhammad Specialist Hospital Kano, Kano State, Nigeria n = 130

Sex	Age	Isolate	Number of Organisms Present (%)
F	AD	*E. coli*	113(86.9)%
		Salmonella	100(76.9)%
		Shigella	95(73.1)%
F	CH	*E. coli*	3(2.3)%
		Salmonella	3(2.3)%
		Shigella	3(2.3)%
M	AD	*E. coli*	*10* (7.7)%
		Salmonella	4(3.1)%
		Shigella	4(3.1)%
M	CH	*E. coli*	4(3.1)%
		Salmonella	2(1.5)%
		Shigella	2(1.5)%

Key: F = Female, M = Male, CH = Children, AD = Adult, N = Total number of samples collected and % = percentage

Discussion

It revealed that the *E. coli*, *Salmonell*a and *Shigella* were isolated from fecal/ diarrheal samples. They were tested under IMVIC; indole, methyl red, vorges proskauer and citrate test. Whereby, *E.coli* and *Salmonella* were indole positive while *Shigella* was the only indole negative isolates. All of the isolates were methyl red positive at the same time vorges proskauer negative and citrate test as well. Moreover, *E. coli*, appeared pinkish but *Salmonella* and *Shigella* appeared whitish and pale in colour morphologically, this is line with the work of (Cheesbrough, 2000).

Conclusion

Many pathogenic bacteria such as *Salmonella* and *Shigella* are transmitted by faeces, contaminated food or water, when such food or water taken in, symptom of diarrhea may develop (Jonas *et al*., 1997). However, the role of normal inhabitant of the gastrointestinal tract in human enteritis has not been clearly understood, the work of (Jonas *et al*., 1997). Moreover, with the help of biochemical identification, such groups of enterobacteriaceas are investigated without much problem.

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