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Isolation of beta-lactamase producing organisms from nasal cavity of students and bike riders

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Abstract
Beta-lactamases are enzymes produced by some bacteria allow in them to provide resistance to beta-lactam antibiotics like Penicillins, Cephamycins, and Carbapenems (Ertapenem), although Carbapenems are relatively resistant to beta-lactamase. The aim of this study is to ascertain the prevalence of beta lactamase producing microorganisms from nasal cavity of students and bike men in Ekpoma, Edo State. The study population of this study comprises of students and bike men in Ekpoma. The subjects are within the age range of 19-48 years. The investigation was carried out on seventy (70) subjects. Specifically, the subjects comprised of twenty (20) students and fifty (50) Bike men in Ekpoma. Specimens to be studied were obtained from the nasal cavity with sterile swabs and plated on the appropriate media. In this study 70 subjects were sampled out of which 50 were bike men and 20 were students in Ekpoma, Edo State. The organisms isolated from the study were, *Haemophilus influenza, Streptococcus* spp and *Staphylococcus aureus.* Among the male subjects, *Staphylococcus aureus, Haemophilus influenzae* and *Streptococcus* spp. were isolate while in the female subjects sampled; *Staphylococcus aureus* and *Haemophilus influenza* were isolated. Subjects within the age range of 19 – 24 years (28) were the highest number sampled with 13 bike men and 15 students while those within the age range of 43 – 48 years (3) were the least sample. *Staphylococcus aureus, Streptococcus* spp and *Haemophilus influenzae* were isolated from subjects within the age range of 19 – 24 years, 25 – 30 years and 31 – 36 years, while *Streptococcus* spp and *Haemophilus influenzae* was isolated from subjects within the age range of 37 – 42 years and 43 - 48 years. Beta-latamase distribution of *Staphylococcus aureus, Streptococcus* spp and *Haemophilus influenzae* was significant in this study.

Keywords: Isolation, beta-lactamase, organisms, *Haemophilus influenzae*

Introduction
Beta-lactamases are enzymes produced by some bacteria allow in them to provide resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems (ertapenem), although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure (Lautenbach *et al.*, 2001; Paterson *et al.*, 2003; Iyevhobu, 2021) [7, 8, 5]. These antibiotics all have a common element in their molecular structure: a four-atom ring known as a beta-lactam. Through hydrolysis, the lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties (Iyevhobu and Obodo, 2021) [6]. Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria (Iyevhobu, Airefetalor, Omolumen, Osagiede, Ikede, Ken-Iyevhobu, Elimian, 2022) [4]. Beta-lactamases produced by Gram-negative organisms are usually secreted, especially when antibiotics are present in the environment used for conventional treatment of bacterial infection (Perez *et al.*, 2007; Iyevhobu and Obodo, 2021) [9, 6].

Currently, ESBLs are becoming a major threat for patients in the hospital, long-term care facilities, and community. These bacteria have not only caused outbreaks but have become endemic in many hospitals throughout the world (Brenwald *et al.*, 1991) [2]. When a significant proportion of Gram-negative isolates in a particular unit is ESBL producers, empirical therapy may change towards use of imipenem, quinolones, or B-lactam/B-lactamase inhibitor combinations (Thomson and Sanders, 1997) [13]. In some centers this has been associated with emergence of imipenem resistance in *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and in ESBL-producing organisms themselves (Lautenbach *et al.*, 2001; Iyevhobu, 2021) [7, 5]. The control of endemic ESBL producers is difficult, and may only be possible after significant nursing and medical reorganization with research, at substantial financial cost (Tenover *et al.*, 2003) [12].

Patients at high risk for developing colonization or infection with ESBL producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present (urinary catheters, endotracheal tubes, central venous lines) for a prolonged duration (Tenover *et al.,* 2003) [12]. A myriad of other risk factors have been implicated including the presence of nasogastric tubes, gastrostomy or jejunostomy tubes and arterial lines, administration of total parenteral nutrition, recent surgery, haemodialysis, decubitus ulcers, and poor nutritional status. Heavy antibiotic use is also a risk factor for acquisition of an ESBL-producing organism (Perez *et al.,* 2007) [9]. A strong relationship exists between third-generation cephalosporin use and acquisition of an ESBL producing strain (Perez *et al.,* 2007) [9]. Other antibiotic classes that have been found to be associated with subsequent infections due to ESBL-producing organisms include quinolones, trimethoprim- sulphamethoxazole, aminoglycosides and metronidazole (Paterson *et al.,* 2003; Tenover *et al.,* 2003) [8, 12].

Beta-lactamases are enzymes produced by bacteria (Iyevhobu *et al.,* 2022) [5], that provide multi-resistance to β-lactam antibiotics such as penicillins, Cephalosporins, cephamycins, and carbapenems (ertapenem), although carbapenems are relatively resistant to beta-lactamase (Surana and Kasper, 2014) [11]. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure (Iyevhobu, 2021) [5]. Microorganisms colonizing the human body are involved in important immunologic processes. They prevent the establishment of potentially harmful pathogens and assist in improving the immune system. On the other hand, the microbiota may promote the development of allergic diseases and is a major reservoir for endogenous infections (Surana and Kasper, 2014) [11]. It is therefore necessary through microbiology laboratory investigation and surveillance to ascertain the prevalence of beta lactamase producing microorganisms in nasal cavity of students and bike men in Ekpoma, Edo State. The aim of this study is to ascertain the prevalence of beta lactamase producing microorganisms in nasal cavity of students and bike men in Ekpoma, Edo State.

Materials and Method

This study was carried out in the Ekpoma, Esan West Local Government Area of Edo State, Nigeria. The study population of this study comprises of apparently students and bike men in Ekpoma. The subjects are within the age range of 19-48 years. The investigation was carried out on seventy (70) subjects. Specifically, the subjects comprised of twenty (20) students and fifty (50) Bike men in Ekpoma. Information was obtained from each participant as regards, marital status, health status as well as any underlying illness. Microbiological investigations were carried out on the samples obtained.

The following materials and apparatus were used for the bacteriological analysis: Nutrient agar, MaCconkay agar, Mueller Hinton agar, sterile universal container, Petri dishes, conical flask, distilled water, autoclave, Bunsen burner, inoculating wire loop, microscope, microscope slide, hollground slide, weighing balance, measuring cylinder, glass slides, Covas reagent, human plasma, hydrogen peroxide, crystal violet Lugol's iodine, acetone, neutral red, Ceftraixone, Clavulinic sensitivity discs and commercially prepared sensitivity disc with quinolones class (Ciprofloxacin, Ofloxacin) and other reagents.

Ethical permission was obtained and informed consent for collection of samples was requested and provided by the subjects through verbal means by educating them on the importance of the research and those that gave their consent will be enlisted for the research. Specimens to be studied were obtained from the nasal cavity with sterile swabs and plated on the appropriate media.

Laboratory Analysis

Culture: This is done to cultivate microorganism from a named sample with appropriate media in the laboratory. The culture media used were both solid and liquid media (Nutrient agar, Macconkay agar, Mueller-Hinton agar and broth). With a standard sterile wire sample was inoculated on each agar surface (primary well). Spreading were done by streaking from the primary inoculum using the standard sterile wire loop flamed at interface to obtain discrete bacteria colonies. The plates were then be incubated at 37 ^{0}C for 24 hours. Bacteria growth was observe after incubation.

Identification of Test Isolates: All isolates in this study colonial morphology will be identify by their colonial appearance on the media which include Size, Shape, Elevation, Opacity, Edge, Colour, haemolysis and fermentation. This was followed by Gram staining.

Methods for Beta-lactamase detection: Method for the preparation of Beta-lactamase reagent: 2g of benzyl penicillin was added to 20ml of phosphate buffer and 0.04g of Bromocresoyl purple was then added and watched for possible reaction.

Acidometric method for detection of Beta-lactamase organisms. Petri dish was placed on working bench, then a filter paper was placed on the petri dish, beta-lactamase reagent which is purple in colour was added to the filter paper and a colony of the organism was added to the beta-lactamase reagent. This was observed for a yellowish colouration which is positive for beta-lactamase e.g *Staphylococcus aureus.* Gram Staining was carried out.

Biochemical Characterisation and Identification

Catalase test was done on Gram positive cocci. Catalase negative Gram positive Cocci in chains were identified as *Streptococcus species* while the catalase positive cocci in clusters were identified as *Staphylococcus* species. Coagulase test was carried out on all the catalase positive cocci. The coagulase positive organisms were identified as *Staphylococcus aureus* while the coagulase negative organism were identify as *Staphylococcus albus.* For the Gram negative bacilli, overnight broth cultures were made for each by adding the colonies to sterilized peptone water and incubated for 24 hours at 37 ^{0}C and motility test was done to ascertain their motility. Among the Gram negative bacilli isolated that were non lactose fermenting, motile and oxidase test was done by placing the filter paper on the petri dish and adding two drops of oxidase reagent (which contain tetramethyl para phenylene diamine dihydrochloride). A glass edge was used to pick the colony from the agar plate and smeared on the moist filter paper with oxidase reagent and was observed for less than 10 seconds for the production of deep blue coloration, which indicates oxidase positive and this biochemical test

confirmed *Pseudomonas species.* The lactose fermenting Gram negative bacilli and motile, Indole test was done by adding some drops of Kovac's reagent (Paradimethylamino benzldehyde) to the overnight broth cultures. Development of red ring at the surface after ten seconds indicates positive reaction and such bacteria were regarded as *Escherichia coli*. For those colonies that were Lactose Fermenters and Oxidase negative, Urease test were performed on them. Having been positive to Urease and negative to Indole confirmed *Klebsiella pneumoniae*.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was performed on Muller Hinton agar as described by The Clinical and Laboratory Standard Institute (2006) [3], (CLSI) on all the microoragnisms isolated by plating inoculated broth on Mueller Hinthon agar, and placing a Gram negative disc, incubated at 37^{O}C for 18- 24 hours, and was read with (CLSI) antibiotics susceptibility pattern.

ESBL Presumptive Test Using Double Disc Synergy Test (DDST)

The DDST performed on Muller Hinton agar as described by Bradford, (2001), on all isolates showing reduced susceptibility and resistance to the third generation cephalosporin (Ceftriaxone and ceftazidine) used to screen for ESBL production. Muller Hinton agar (Oxiod, U.K) was inoculated from a blood agar plate grown overnight as recommended for the disc diffusion test (Tenover *et al.*, 2003) [12]. An Amoxicillin Clavulanate disc was placed at the center of the incubated plates and the disc containing the standard 30ug of Ceftazidine, Cefotamine and Ceftriazone were placed 20mm apart from the Amoxicillin-clavulanate disc.

Results

This study evaluates Beta-lactamase producing organisms from nasal cavity of students and bike men in Ekpoma. Table 1 shows the organisms isolated from the study. The isolates were *Staphylococcus aureus* and *Streptococcus* spp.

Table 2 shows the relationship between gender and organisms isolated. Among the male subjects, *Staphylococcus aureus, Haemophilus influenzae* and *Streptococcus* spp. were isolate while in the female subjects sampled, *Staphylococcus aureus* and *Haemophilus influenza* were isolated.

Table 3 shows the relationship between age range and subjects examined in the study. Subjects within the age range of 19 – 24 years (28) were the highest number sampled with 13 bike men and 15 students while those within the age range of 43 – 48 years (3) were the least sample.

Table 4 shows the Samples and Organisms isolated according to age range. *Staphylococcus aureus, Streptococcus* spp and *Haemophilus influenzae* were isolated from subjects within the age range of 19 – 24 years, 25 – 30 years and 31 – 36 years, while *Streptococcus* spp and *Haemophilus influenza* was isolated from subjects within the age range of 37 – 42 years and 43 - 48 years.

Table 1: Organisms Isolated in the study

Samples	Organisms Isolated
Students	*Streptococcus* spp.
Bike men	*Staphylococcus aureus*, *Streptococcus* spp.

Table 2: Relationship between gender and organisms isolated

Gender	Organisms Isolated
Male	*Staphylococcus aureus*, *Streptococcus* spp., *Haemophilus influenzae*
Female	*Staphylococcus aureus*, *Haemophilus influenzae*

Table 3: Relationship between age range and subjects examined in the study

Age range	Bike men (%)	Students (%)
19 – 24	12 (24.0)	15 (75.0)
25 – 30	18 (36.0)	5 (25.0)
31 – 36	10 (20.0)	0 (0.0)
37 – 42	7 (14.0)	0 (0.0)
43 - 48	3 (6.0)	0 (0.0)
TOTAL	50 (100)	20 (100)

Table 4: Samples and Organisms isolated according to age range

Age Range	Organisms Isolated
19 – 24	*Staphylococcus aureus*, *Streptococcus* spp., *Haemophilus influenzae*
25 – 30	*Staphylococcus aureus*, *Streptococcus* spp., *Haemophilus influenzae*
31 – 36	*Staphylococcus aureus*, *Streptococcus* spp., *Haemophilus influenzae*
37 – 42	*Streptococcus* spp., *Haemophilus influenzae*
43 – 48	*Streptococcus* spp., *Haemophilus influenzae*

Table 5: Antibiotic Susceptibility Testing

Antibiotics	Organisms		Antibiotics	Organisms
Gram Positive Disc	***Staphylococcus aureus***	***Streptococcus* spp.**	**Gram Negative Disc**	***Haemophilus influenzae***
Gentamycin	+	+	Streptomycin	+
Chloramphenicol	+	-	Ciprofloxacin	+
Ciprofloxacin	+	+	Gentamycin	+
Erythromycin	+	+	Levofloxacin	-
Levofloxacin	+	+	Cefuroxin	-
Ampiclox	+	-	Azithromycin	+
Rifampicin	+	+	Ceftriaxone	-
Norfloxacin	-	-	Erythromycin	+
Streptomycin	-	+	Chloramphenicol	+
Amoxil	+	+	Ampicillin	+

Table 6: Cultural Characteristics and Biochemical Analysis Bacterial Isolates

Organism	Cultural characteristics					Biochemical analysis							
	Shape	Elevation	Opacity	Consistency	Colour	Gram	Catalase	Coagulase	Indole	Motility	Oxidase	Citrate	Urease
Staphylococcus aureus	Cocci in cluster	Spherical	Translucent	Moist	Pale	+	+	+	-	-	-	+	+
Haemophilus influenzae	Cocco bacilli	Flat	Opaque	Mucoid	Colourless	-	+	-	+	-	+	-	+
Streptococcus spp.	Diplococci	Flat	Opaque	Moist	Green	+	-	-	-	-	-	+	-

Key
+ = Positive,
- = Negative

Discussion

In this study 70 subjects were sampled out of which 50 were bike men and 20 were students in Ekpoma, Edo State. The organisms isolated from the study were, *Haemophilus influenza, Streptococcus* spp and *Staphylococcus aureus.* Among the male subjects, *Staphylococcus aureus, Haemophilus influenzae* and *Streptococcus* spp. were isolate while in the female subjects sampled; *Staphylococcus aureus* and *Haemophilus influenza* were isolated. Subjects within the age range of 19 – 24 years (28) were the highest number sampled with 13 bike men and 15 students while those within the age range of 43 – 48 years (3) were the least sample. *Staphylococcus aureus, Streptococcus* spp and *Haemophilus influenzae* were isolated from subjects within the age range of 19 – 24 years, 25 – 30 years and 31 – 36 years, while *Streptococcus* spp and *Haemophilus influenzae* was isolated from subjects within the age range of 37 – 42 years and 43 - 48 years.

The origin of organisms that are introduced into the sinuses and may eventually cause sinusitis is the nasal cavity. The normal flora of that site includes *Staphylococcus aureus, Staphylococcus epidermidis,* a- and *g-streptococci, Propionibacterium acnes,* and aerobic diphtheroid. The main mechanism of resistance observed among the invasive *H. influenzae* isolates analysed was b-lactamase production (Stenutz *et al.*, 2006; Iyevhobu, 2021) [, 5]. Beta-latamase distribution of *Staphylococcus aureus, Streptococcus* spp and *Haemophilus influenzae* was significant in this study.

Conclusion

In conclusion from this research, Beta-latamase producing organisms we found in the subjects studied. In conclusion, *Staphylococcus aureus* and other bacteria were abundant in the posterior cavity, suggesting that the topical medicine should be applied to the whole nasal cavity.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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