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PREVALENCE OF β-LACTAMASE PRODUCTION AMONG PATHOGENIC BACTERIA ISOLATED FROM SURGICAL SITE AND WOUND INFECTION AMONG PATIENTS ADMITTED IN SOME SELECTED HOSPITALS IN SOKOTO METROPOLIS, NIGERIA

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Abstract

Antimicrobial resistance among pathogenic bacteria is increasing worldwide especially against ß-lactam drugs, due to the production of ß-lactamase enzymes which destroy the ß-lactam ring of these antibiotics, thus preventing the action of penicillin binding proteins (PBPs). The prevalence of β-lactamase producing bacteria among patients admitted in three different hospitals were carried out in this study. The results of this study shows that out of one hundred and fifty one isolates obtained in three different hospitals in Sokoto metropolis, only 82 (54.0%) were resistant to the antibiotics tested. These include 42 (51.2%) were isolated in Usmanu Danfodiyo Teaching Hospital (UDUT), 26 (31.7%) were isolated from Specialist Hospital Sokoto (S.H.S) and 14 (17.1%) were isolated from Maryam Abatcha Women and Children Hospital (MAWCH) which has the least number of occurrence of the resistant isolates. β-lactamase test was carried out on the resistant isolates show s that out of the 82 isolates found resistant to the antibiotics tested, about 60 (73.2%) were β- lactamase positive and the remaining 22 (26.8%) were β-lactamase negative. *Staphylococcus aureus* has the highest resistant bacteria producing β-lactamase enzyme with 22 isolates, followed by *Proteus mirabilis* with 10 isolates.

Key words: β-lactamase, Prevalence, UDUTH, S.H.S and MAWCH

Introduction

Antimicrobial resistance among pathogenic bacteria is increasing worldwide especially against ß-lactam drugs, due to the development of ß-lactamase enzymes (NNIS System, 2003) which destroy the ß-lactam ring of these antibiotics, thus preventing the action of penicillin binding proteins (PBPs) (Livermore, 1995). The antibacterial agents become so changed in their chemical structure that they are no longer recognized by the enzymes responsible for making the Peptidoglycan layer of the bacterial cell wall (Aggarwar and Chaudhry, 2004). Of the various mechanisms of acquired resistance to β-lactam antibiotics, resistance due to β -lactamases is the most prevalent. Gram negative bacteria resistant to agents such as extended spectrum cephalosporin, monobactams, carbapenems and β-lactam--lactamase inhibitor combinations have emerged through the production of a variety of β–lactamases (Pitout *et.al*, 2010) Emergence of resistance to these agents has resulted in a major clinical crisis. This problem also leads to wastage of precious time of patients and a great drawback economically. Bacterial infections associated with multi-drug resistance in cancer patients have been reported as high and this is caused mostly by the effect of the cytotoxic chemotherapy and radiotherapy which lowers the immunity (Rice *et.al*, 1990).

Beta-lactamase producing *Enterobacteriaceae* has become a global problem. The rate of incidence and type of beta-lactamase enzyme varies in different worldwide geographical locations. From more than 30 countries, research related to Extended Spectrum Beta-lactamases (ESBLs) has been published which presents worldwide distribution of ESBLs (Paterson and Bonomo, 2005; Coque *et al.,* 2008). Beta-lactamase producing bacteria have property of enzyme production and it is reported that if not properly diagnosed inappropriate antibiotics pattern results. Beta-lactamase producing *Enterobacteriaceae* has become a global problem. The rate of incidence and type of beta-lactamase enzyme varies in different worldwide geographical locations. Advances in control of infections have not eliminated the risk of post-operative wound infections due to emergence and spread of resistant microbes. The condition is serious particularly in developing countries where irrational prescription of antimicrobial agents is common. Measures including new antimicrobial production, better infection control program and rational use of existing antimicrobial agents have been suggested to reduce the problem (Anguzu and Olila, 2007; Andhoga *et al*., 2002; Hart and Kariuki, 1998). Thus, the aim of this research work is to determine the prevalence of pathogenic bacteria producing β-lactamase enzymes

among patients with surgical and open wound admitted in some selected hospitals in Sokoto metropolis, Nigeria.

Materials and method

Study area

The study was carried out in Sokoto metropolis, Sokoto state, Nigeria. It was located between longitudes 40 to 60 40' north and it covers approximately an area of 56,000 square kilometer. Prior to the creation of Zamfara state the population of Sokoto state was estimated at about 4,392,391 million. There exist within the state different health facilities at tertiary, secondary and primary levels including comprehensive health center, women and children welfare clinic and upgraded dispensaries. The people of Sokoto are subsistent famers, agriculture is predominantly the occupation of the inhabitants especially those in the rural area (Hauwa *et al*, 1997). The state is endowed with livestock resources: indeed the state is rated second with regard to livestock population. There are two major seasons, namely wet and dry. The dry season start from October to April but in some parts, it may extend to May. The wet season begins in May to September (Anon, 2001).

Sample Collection

Patients with surgical and open wounds attending Specialist hospital Sokoto (SHS), Usmanu Danfodio University Teaching Hospital (UDUTH) and Maryam Abacha Women and Children Hospital Sokoto (MAWCH) were sampled. The hospitals were situated in Sokoto metropolis; the choice was based on the fact that the hospitals were one of the centers which attend to large number of patients from different socio economic backgrounds in the state. Consent of the hospital management and patient were sought for prior to sampling. A total of 200 samples of patient were collected, of which 80 samples were from SHS, 80 also from UDUTH and 40 samples from MAWCH. From the total samples, 123 were males and 77 were females with surgical and open wounds using sterile commercial swab sticks

Sensitivity Tests

Sensitivity, screening and confirmatory sensitivity testing was carried out on the isolates using disc diffusion method. Bacterial growth was standardized by comparing with McFarland turbidity standard as follows:-

Preparation of McFarland turbidity standard

One percent (1%) v/v solution of sulphuric acid solution was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. A quantity of 1% w/v of barium chloride solution was prepared by dissolving 0.5g of dehydrate barium chloride in 50ml of distilled water. 0.06ml of barium chloride solution was added to 99.4ml of sulphuric acid solution and mixed. Ten tubes of the turbid solution of different concentration was prepared, same type as used for preparing the test and control inoculum (Cheesbrough, 2002).

Preparation of test inoculum

Using a sterile wire loop, 3-5 well isolated colonies of the isolates were emulsified in 3-4ml of sterile physiological saline or nutrient broth. In a good light the turbidity of the suspension was matched with that of the ten McFarland standard prepared, and a match was selected and used for the test.

Test for Beta – lactamase Production

Staphylococcus aureus, Coagulase-negative *Staphylococci*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Proteus mirabilis*, *Klebsiella* spp, *Psuedomonas aeruginosa*, *Citrobacter fruendii* and *Eschericia coli* identified were tested for the presence of B–Lactamase enzyme using iodometric method. Starch agar (nutrient agar containing 2% soluble starch) was inoculated with the test organism. The surface of the overnight culture was flooded with freshly prepared 10,000 unit/ml (6mg/ml) of benzyl penecillin and left for sixty minutes at room temperature. Thereafter iodine solution was added. Isolate whose colonies turned blue-black with colourless halos were considered as beta lactamase producing isolates and recorded (Crosa *et al*., 1994).

Result

The results of this study shows that out of one hundred and fifty one isolates obtained in three different hospitals in Sokoto metropolis, only 82 (54.0%) were resistant to the antibiotics tested. These include 42 (51.2%) were isolated in UDUT, 26 (31.7%) were isolated from Specialist Hospital Sokoto and 14 (17.1%) were isolated from MAWC Hospital and also had the least number of resistant isolates. Also, among the isolates, *Staphylococcus aureus* was most frequent pathogen isolated in the hospitals which also possess considerable amount of resistance,

followed by Coagulase negative *Staphylococcus, Proteus mirabilis* and *Pseudomonas species* (Table 1).

β-lactamase test was carried out on the resistant isolates showed that, of the 82 isolates found resistant to the antibiotics tested, about 60 (73.2%) were β- lactamase positive and the remaining 22 (26.8%) were β-lactamase negative. *Staphylococcus aureus* was the most prevalent resistant bacteria producing β-lactamase enzyme with 22 isolates, followed by *Proteus mirabilis* with 10 isolates that are β-lactamase producers, although, highly statistical significance difference was observed between β-lactamase positive and β-lactamase negative ($t = 3.6819$, $df = 17$, $p = 0.001849$) (Table 2)

Table 1: Frequency of occurrence of the resistant bacteria isolates

Bact. Isolated	**Total isolates (%)**	**No. of Resistant isolates**	**UDUTH**	**MAWCH**	**S. H. S (%)**
Staph aureus	54(35.76)	28 (51.8%)	14 (50.0)	4 (14.3)	10 (35.7)
Coa neg Staph.	47(31.1)	16 (34.0%)	8 (100)	5 (31.2)	3 (18.8)
Strept pyogene.	7(4.64)	4 (57.1%)	2 (50.0)	1 (25.0)	1 (25.0)
Proteus v.	9(5.96)	6 (66.6%)	3 (50.0)	0 (0.0)	3 (50.0)
Proteus m.	13(8.61)	12 (92.3%)	6 (50.0)	1 (8.3)	5 (41.7)
Klebsiela spp.	5(3.31)	2 (40.0%)	1 (55.6)	0 (0.0)	1 (50.0)
Pseudomonas	9(5.96)	9 (100%)	5 (55.6)	2 (22.2)	2 (22.2)
E.coli	5(3.31)	3 (60%)	1 (33.3)	1 (33.3)	1 (33.3)
Citrobacter	2(1.32)	2 (100%)	2 (100)	0 (0.0)	0 (0.0)
Total	**151(100)**	**82 (54%)**	**42 (51.2)**	**14 (17.1)**	**26 (31.7)**

Table 2: Beta-lactamase test of the resistant bacteria

Bacteria isolates	**B-Lactamase +ve (%)**	**B-Lactamase -ve (%)**
Staph aureus	22(78.5)	5(17.8)
Coag neg *Staph.*	9(56.3)	8(50.0)
Strept pyogenes.	3(75.0)	1(25.0)
Proteus vulgaris	3(50.0)	3(50.0)
Proteus mirabilis	10(83.3)	2(16.6)

Klebsiella spp.	2(100)	0(0.0)
Pseudomonas.	6(66.6)	3(33.3)
Escherichia coli	3(100)	0(0.0)
Citro freundii.	2(100)	0(0.0)
Total	**60(73.2)**	**22(26.8)**

(t = 3.6819, df = 17, p = 0.001849)

Discussion

The emergency of antibiotic resistance among pathogenic bacteria has become apparent in most of our hospital environments and it is matter of great concern. This may results due to misuse of antibiotic by our clinicians which result in the production of β-lactamase enzymes. Out of one hundred and fifty one isolates identified in this study only 82 (54.0%) were resistant to the antibiotics tested. This includes *Staphylococcus aureus* (28), *Coag neg Staph* (16), *P. mirabilis* (12), *Pseudomonas aeruginosa* (9), *Prot. vulgaris* (6), *Strept pyogenes* (4), *Escherichia coli* (3), *Klebsiella species* and *Citrobacter freundii* (2). *Staphylococcus aureus* is the highest isolate in this study; this could be due to the fact that it is normal flora of the skin. This work agrees with that of Azene and Beyene (2011) who reported that *S. aureus* had the highest frequency of occurrence among patients wound in Tanzania. Also it is the most prevalent organism isolated in the study of Bhat and Vasaikar, (2011) which account for 27.7%. Conversely, the result is not in line with the work of Beheshti and Zia (2011) who reported that *Pseudomonas aeruginosa* was the most common organism (32.2%) isolated among patients wound in Iran. The presence of *Staphylococcus aureus* in this study could possibly play a key role in wound infections in the identified area. The implication of this finding is that *Staphylococcus aureus* is a successful pathogen that has combination of bacterial immuno-evasive strategies. One of these strategies is the production of carotenoid pigment staphyloxanthin, which is responsible for the characteristic golden colour of *S. aureus* colonies. This pigment acts as a virulence factor, primarily by being a bacterial antioxidant which helps the microbe evade the reactive oxygen species which the host immune system uses to kill pathogens (Clauditz *et al.*, 2006; Liu *et al.*, 2005) (Table 1).

β- lactamase test of the resistant isolates were carried out in this study. Sixty 60 (73.2%) isolates were found to produce β-lactamase, this includes *Staphylococcus aureus* 22 (78.5%), *P. Mirabilis* 10 (83.3%), *Coag neg Staph* 8 (50.0%), *Pseudomonas aeruginosa* 6 (66.6%), the least among the isolates were *Klebsiella species* and *Citrobacter freundii* 2 (100%). This can be related to the previous result (Table 2) that the resistance could be due to their ability to produce enzymes such as β-lactamase. β-lactamase acts on β-lactam ring of penicillins and penicillins derivatives rendering the drug inactive (Brook, 1984). *Staphylococcus aureus* was the most prevalent bacteria producing β-lactamase enzymes. The implication of the wound infections caused by these organisms isolated in this study is that they make treatment expensive, since it could not be possible with β- lactam antibiotic. The result of this study similar to the work of Tesfahunegn *et al.* (2009) who showed that *Staphylococcus aureus* was the most prevalent resistant organism isolated from wound samples among patients attending hospitals in Ethiopia.

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