ANTIBIOGRAM AND DETECTION OF BETA LACTAMASE PRODUCTION AMONG MICROORGANISMS ASSOCIATED WITH NOSOCOMIAL INFECTIONS

[1]Ugbo E. N., [*1]Moses I. B., [1]Iroha I. R, [2]Ilang D. C., [3]Egbule C. U., [4]Eluu S. C., [1]Uzoeto H. O.

[1,*1]Department of Applied Microbiology, Ebonyi State University, Faculty of Sciences, Ebonyi State University, Abakaliki.

[2]Department of Biological Science, Federal University Ndufu-Alike, Ikwo, Ebonyi State, Nigeria.

[3]Department of Science Laboratory Technology, Akanu-Ibiam Federal Polytechnic, Unwana, P. M. B. 1007, Ebonyi State.

[4]Department of Biotechnology, Faculty of Sciences, Ebonyi State University, P. M. B. 053 Abakaliki, Ebonyi State, Nigeria.

Abstract

Nosocomial infections are one of the leading causes of death and morbidity among hospitalized patients. This study phenotypically evaluated the bacterial contamination rate of hospital equipment as sources of nosocomial infections and detection of beta-lactamase production among the isolates. A total of 30 swab samples from hospital equipment and other fomites collected from Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria were obtained for this study. The samples were bacteriologically analyzed in the microbiology laboratory unit of Ebonyi State University, Abakaliki and the isolates were identified using standard microbiological techniques. Antimicrobial susceptibility test was performed using the Kirby-Bauer disk diffusion technique. Beta-lactamase production was detected in the bacterial isolates using nitrocefin stricks (Oxoid, UK). A total of 23 bacterial isolates which includes *S. aureus* 10(43.5 %), *E. coli* 9(39.1 %), *P. aeruginosa* 2(8.7 %) and *K. pneumoniae* 2(8.7 %) were recovered from the 30 swab samples. Notably, bacterial isolates in this study showed different degrees of susceptibility and resistance to the tested antibiotics. *S. aureus* isolates were highly resistant to ceftazidime (90.0 %), cefotaxime (90.0 %), ceftriaxone (90.0 %), cefpodoxime (100.0 %), aztreonam (80.0 %), augmentin (60.0 %) and cefepime (50.0 %). *E. coli* isolates were resistant to ceftazidime (55.6 %), cefotaxime (55.6 %), cefpodoxime (55.6 %), aztreonam (55.6 %), cefepime (44.4 %) and augumatin (55.6 %). However, *P. aeruginosa* and *K. pneumoniae* exhibited complete resistance (100 %) to ceftazidime, cefotaxime and cefpodoxime. Only 11 (57.9 %) bacterial isolates (*S. aureus* (4(17.4 %)), *E. coli* (4(17.4 %)), *P. aeruginosa* (1(4.4 %)) and *K. pneumoniae* (2(8.7 %)) were phenotypically confirmed to produce beta-lactamase enzymes. Constant decontamination of hospital environments, equipment and fomites used in the housing and treatment of patients attending hospitals can help to prevent the spread of antibiotic resistant bacteria, and thus preserve the efficacy of available antibiotics.

Keywords: Nosocomial infections, Hospital equipment, Antibiotic resistance, Beta-lactamase

Introduction

Nosocomial infections (NI) also known as hospital associated/acquired infections (HAI) are those infections that develop in a patient during his/her stay in a hospital or other types of clinical facilities which were not present at the time of admission (Chikere *et al*., 2008). Hospital-acquired infections (HAI) have been recognized for over a century as a critical problem affecting the quality of healthcare, and they constitute a major source of adverse healthcare outcomes; this affects 1 in 10 patients admitted to hospital (Aly *et al*., 2008). Patients in intensive care units (ICUs) are at high risk of developing nosocomial infections as a result of mechanical use of invasive procedures and their immuno-compromised status (Prakash, 2001). Nosocomial infections can be spread to susceptible patients in the clinical setting by a number of means. Healthcare workers can spread this infection through contaminated fomites and other hospital equipment such as thermometer, sphygmomanometer, stethoscope, folder, bedrail, air droplets, work bench and taps (Bukharia *et al*., 2004; Eltablawy and Elhifinawi, 2009; Tagoe and Debordes, 2012). Contamination of environmental objects and surface is a common phenomenon and literature has reviewed that in human environment, microorganisms colonize and contaminate environmental objects in home and hospital (Brady *et al.,* 2007). In some cases, the microorganism originates from the patient's own skin microbiota, becoming opportunist after surgery or other procedures that compromise the protective barrier. Nosocomial infection cause severe pneumonia and urinary tract infection. It may become clinically apparent either during the hospitalization or after discharge (Haubler, 2007). However, an asymptomatic patient may be considered infected if pathogenic microorganisms are found in a body fluid or at a body site that is normally sterile, such as the cerebrospinal fluid or blood (Chikere *et al*., 2008). Infections acquired in hospitals have existed since the very inception of hospitals themselves, and continue to be an important health problem even in the modern era of antibiotics. They result in high morbidity and mortality, extended hospitalization, greater use of antibiotics, and increased costs (Blot *et al.,* 2003). Studies have indicated that nosocomial infections occurred in 5 % - 10 % of all hospitalizations in Europe and North America and in more than 40 % of hospitalizations in parts of Asia, Latin America, and sub-Saharan Africa (WHO, 2002). Bacteriologically, almost any organisms have the potential to cause nosocomial infection but only limited numbers of organisms are frequently isolated. Coagulase-negative staphylococci are isolated almost twice as often as *S. aureus*. *Enterococcus* species is frequently isolated from surgical site infection (SSI), and blood stream infection (BSI) but rarely found in the respiratory

tract. *Pseudomonas aeruginosa* is isolated from about 1/10 of all infections and appears to evenly affect all of the major sites except the blood stream, where it is found less often (Murray *et al*., 2005). Large usage of broad-spectrum antibiotics in hospital environment promotes emergence and reemergence of difficult-to-treat nosocomial infections in patients. Methicillin resistant *S. aureus* (MRSA) and Gram-negative bacilli emerged as agents responsible for nosocomial infections. The emergence of multidrug resistant Gram-negative bacilli, *Pseudomonas* species and *Acinetobacter* species was observed, presenting difficult therapeutic problems (Rachael *et al.,* 2008; Blot *et al.,* 2003). Incidence of *Escherichia coli* and *Klebsiella pneumoniae* and simultaneous persistence of *Pseudomonas aeruginosa*, *Acinetobacter* species and emergence of newer nosocomial Gram-negative organisms such as *Burkholderia cepacia* and *Stenotrophomonas maltophilia* has been reported (Klein *et al*., 2007). Nosocomial infections are the major cause of death and increased morbidity in hospitalized patients. They may cause increased functional disability and emotional stress and may lead to conditions that reduce quality of life. The study of nosocomial infections has its origin in the 18th century. Nevertheless, organ transplantation, blood transfusion and hospital equipment and materials are also recognized as other causes of nosocomial infections. *Staphylococcus aureus, Staphylococcus epidermidis,* Gram negative bacilli, *Enterococcus* species, *Candida* Species, *Klebsiella pneumoniae*, *Enterobacter* Species, and *pseudomonas* species have been the most frequently identified causative agents of nosocomial infections (Waterer and Wunderink, 2001). Resistance to third generation cephalosporins was also reported in *E. coli, K. pneumonia* and *Enterobacter* species (Haddadin *et al.,* 2002). As a result of these, our study is focus on investigating the rate of bacterial contamination of hospital equipment as sources of nosocomial infections and detection of beta lactamase production.

Materials and Methods

Sample collection and processing: A total of 30 swab samples were collected from hospital equipment or fomites which include clutches, stethoscope, wheel chair, sphygmomanometer, thermometer, door handle, drip stand and drug desk used in Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria using sterile swab sticks. This was done by rotating the moist sterile swab sticks on the surfaces of the equipment at an angle of 180 ^{0}C. The swab sticks were returned to their respective containers and labeled. They were then transported to the microbiology laboratory unit of Ebonyi State University, Abakaliki for bacteriological analysis. Each of the collected swab samples was inserted into 5 ml of

freshly prepared nutrient broth and incubated incubated at 37 ^{0}C for 18-24 hours. Bacterial growth was identified by the presence of turbidity or cloudiness in the tubes after incubation.

Culture: A loopful of the turbid growth in the tubes was aseptically inoculated onto freshly prepared mannitol salt agar, MacConkey agar, nutrient agar, peptone water and eosin methylene blue (EMB) agar plates. The plates were properly labeled and incubated at 37 ^{0}C for 18- 24 hours. Culture plates that showed bacterial growth were sub-cultured onto freshly prepared macConkey agar, mannitol salt agar, nutrient agar and EMB agar plates for the isolation of pure cultures of *Escherichia coli*, *S. aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* prior to biochemical tests. All the suspected bacterial isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test and other biochemical tests which include oxidase test, indole test, citrate utilization test test, voges proskauer test, methyl, H_2S production red test, urease test and sugar fermentation test (Cheesbrough, 2010).

Antibiotic susceptibility test: This was done on Mueller Hinton agar plates (Oxoid, UK) using the Kirby-Bauer disk diffusion method as per the criteria of Clinical Laboratory Standard Institute (CLSI, 2015). The standard antibiotic discs used include: ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefpodoxime (10 µg), aztreonam (30 µg), cefepime (30 µg), and augumatin (30 µg) (Oxoid, UK). A loopful of the test organism (adjusted to 0.5 McFarland turbidity standards) was streaked on freshly prepared Muller-Hinton agar plates; and the plates were allowed to stand for 15 minutes. The antibiotic discs were placed at a distance of 30 mm apart from one another and 15 mm away from the edge of the culture plate and later incubated at 37 ^{0}C for 24 hours (Ugbo *et al*., 2016; CLSI, 2015). The zones of inhibition were measured and interpreted according to the CLSI criteria (CLSI, 2015).

Detection of beta-lactamase production: *E. coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus* strains isolated in this study were screened for Beta-lactamase production using nitrocefin stricks (Oxoid, UK). Nitrocefin is a chromogenic cephalosporin (incorporated in the nitrocefin test sticks) that changes colour from yellow to red on hydrolysis when the stick comes in contact with a positive test isolate. The colour coded end of the nitrocefin stick was used to touch the bacterial colony and rotated to pick mass of the cells. Two drops of distilled water was used to moisten the tip of the stick and then observed for pink-red colour development after 5-15 minutes (CLSI, 2015; Rosamund *et al*., 1984).

Results

Table 1 shows the frequency of the bacterial isolates from hospital equipment. A total of 30 swab samples from hospital equipment and fomites collected from Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria were used for this study. A total of 23 bacterial isolates which includes 10(43.5 %) *S. aureus*, 9(39.1 %) *E. coli*, 2(8.7 %) *P. aeruginosa* and 2(8.7 %) *K. pneumoniae* were recovered from the 30 hospital equipment and fomite swab samples after bacteriological analysis. Table 2 shows the antibiotic sensitivity profiles of the bacterial isolates (*S. aureus*, *E. coli, P. aeruginosa* and *K. pneumoniae*) to some commonly used antibiotics. Notably, bacterial isolates in this study showed different degrees of susceptibility and resistance to the tested antibiotics. *S. aureus* isolates was found to be highly resistant to ceftazidime (90.0 %), cefotaxime (90.0 %), ceftriaxone (90.0 %), cefpodoxime (100.0 %) and aztreonam (80.0 %) and augmentin (60.0 %). *E. coli* isolates were moderately resistant to ceftazidime (55.6 %), cefotaxime (55.6 %), cefpodoxime (55.6 %), aztreonam (55.6 %) and augmentin (55.6 %). However, *K. aeruginosa* and *P. pneumoniae* exhibited complete resistance (100 %) to ceftazidime, cefotaxime and cefpodoxime. A total of 23 bacterial isolates were screened for beta-lactamase production. Only 11 (47.9 %) bacterial isolates (4(17.4 %) *S. aureus*, 4(17.4 %) *E. coli*, 2(8.7 %) *K. pneumoniae* and 1(4.4 %) *P. areuginosa* were phenotypically confirmed to produce beta-lactamase enzymes in this study (Table 3).

Table 1: Frequency of the bacterial isolates from hospital equipment

Organism	No. of positive samples	% positive samples
Staphylococcus aureus	10	43.5 %
Escherichia coli	9	39.1 %
Pseudomonas aeruginosa	2	8.7 %
Klebsiella pneumoniae	2	8.7 %
Total	23	-

Table 2: Antibiotic sensitivity profiles of the bacterial isolates

Antibiotics (µg)	*S. aureus*		*E. coli*		*P. aeruginosa*		*K. pneumoniae*	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ceftazidime (30)	1(10.0)	9(90.0)	4(44.4)	5(55.6)	0(0.0)	2(100.0)	0(0.0)	2(100.0)
Cefotaxime (30)	1(10.0)	9(90.0)	4(44.4)	5(55.6)	0(0.0)	2(100.0)	0(0.0)	2(100.0)
Ceftriaxone (30)	1(10.0)	9(90.0)	2(22.2)	7(77.8)	1(50.0)	1(50.0)	1(50.0)	1(50.0)
Cefpodoxime (10)	0(0.0)	10(100)	4(44.4)	5(55.6)	0(0.0)	2(100.0)	0(0.0)	2(100.0)
Aztreonam (30)	2(20.0)	8(80.0)	4(44.4)	5(55.6)	1(50.0)	1(50.0)	1(50.0)	1(50.0)
Cefepime (30)	5(50.0)	5(50.0)	5(55.6)	4(44.4)	1(50.0)	1(50.0)	1(50.0)	1(50.0)
Augumatin (30)	6(60.0)	4(40.0)	4(44.4)	5(55.6)	1(50.0)	1(50.0)	1(50.0)	1(50.0)

Table 3: Prevalence of beta-lactamase positive organisms

Organisms	**No of isolates screened**	**Beta-lactamase producers**
S. aureus	10	4 (17.4 %)
E. coli	9	4 (17.4 %)
P. aeruginosa	2	1 (4.4 %)
K. pneumoniae	2	2 (8.7 %)
Total	23	11 (47.9 %)

DISCUSSION AND CONCLUSION

Nosocomial pathogens have the potential to cause severe morbidity and mortality. Microorganisms such as *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aurues*, and *Pseudomonas aeruginosa* are gaining renewed interest because of the emergence of multidrug resistance strains. They represent one of the major threats to effective management of patients in hospitals (Abreu *et al*., 2014). A total of 30 samples collected from different hospital equipment in different wards were used in our study. Out of these samples, 10(43.5 %) *Staphylococcus aureus* and 9(39.1 %) *Escherichia coli* were isolated, whereas the same number of 2(6.67%) *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* each was isolated (table 1). This result corresponds to the report of Orji, 2005, who isolated similar pathogens from hospital equipment but different from the report of Njoku-Obi and Ojieegbe (1993) who did not isolate

any bacterial pathogens in their study involving hospital fomites. Antibiotic sensitivity profile results showed that *S. aureus* isolates were highly resistant to ceftazidime (90.0 %), cefotaxime (90.0 %), ceftriaxone (90.0 %), cefpodoxime (100.0 %), aztreonam (80.0 %) and augmentin (60.0 %). The high level of resistance observed among the *Staphylococcus aureus* isolates in our study could be as a result of these isolates harbouring resistance genes or enzymes such as beta-lactamase enzymes. Also, the site of isolation of the organisms could be a contributing factor. *E. coli* isolates were resistant to ceftazidime (55.6 %), cefotaxime (55.6 %), cefpodoxime (55.6 %), aztreonam (55.6 %), and augmentin (55.6 %). This finding disagrees with the work of Weber *et al*. (2013) and Judge *et al.* (2013) who independently reported that Enterobacteriaceae strains exhibited antibiotic resistance frequencies within the range of 22.2 % on hospital equipment from intensive Care Unit (ICU) beds. The *K. pneumoniae* and *P. aeruginosa* isolated in this study demonstrated high level of resistance to some of the antibiotics tested. In this study, *K. pneumoniae* and *P. aeruginosa* exhibited 100 % resistant to ceftazidime, cefotaxime and cefpodoxime. The drug resistance pattern of *Pseudomonas aeruginosa* described in this study is in total disagreement with the report of Pathmanathen *et al.* (2009) who reported that majority of the antibiotics tested on *Pseudomonas* species isolated from biological materials were very effective against most of the isolated strains. The reasons for some of the variations in results could be as a result of differences in the regions or countries where the work was done; level of the **cleanliness** of the hospital equipment, environment and the level of abuse of these antibiotics. The presence of *Pseudomonas aeruginosa* isolated in this study is similar to the result obtained in the analysis of equipment from different hospital environment as reported by Abreu *et al*. (2014) and Petignant *et al.* (2006) who independently studied *Pseudomonas aeruginosa* persistence in hospital environment and clinical samples. Out of the 23 bacterial isolates screened for beta-lactamase production in this study, 11 (47.9 %) bacterial isolates (4(17.4 %) *S. aureus*, 4(17.4 %) *E. coli*, 2(8.7 %) *K. pneumoniae* and 1(4.4 %) *P. aeruginosa* were phenotypically confirmed to produce beta-lactamase enzymes (Table 3). The result obtained in this work agreed with the work of Manikandan *et al.* (2011) who reported multidrug resistance in bacteria isolated from hospital equipment. Occurrence of beta lactamase-producing bacteria as reported in this study is in line with the previous work done by Uchenna (2005). Our result also agreed with the work of Gales *et al*. (2001) who reported the presence of drug resistance in *P. aeruginosa* isolated from hospital equipment. Harbottle *et al*. (2006) reported that the overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistance

strains of bacteria. From this study, it can be established that different objects or equipment used in hospitals associates directly or indirectly in various degrees to the dissemination of known bacterial pathogens since these equipment can harbour these organisms. The direct role played by these fomites in the transmission of disease was reviewed in this study. The isolation of the following bacterial pathogens: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* presents a serious concern for possible nosocomial transmission. Some researchers concluded in their study that the common nosocomial pathogens may survive well or persist on surface for months and can therefore be a continuous source of transmission if regular surface disinfection is not performed (Kramer, 2006). Although a researcher remarks that inanimate environment has little relevance to the spread of infection; other researchers noted that fomites are involved in the transmission of pathogens in healthcare environments (Ayeliffe, 1991; Prescott *et al*., 2006). Therefore, this research work has reviewed the possibility of hospital equipment or fomites such as clutches, stethoscope, wheelchair, sphygmomanometer, thermometer, door handle, drip stand, drug desk and others used in the hospital environment to be a source of pathogenic bacteria which has the ability to cause nosocomal infections among patients and staffs in hospitals. Notably, Some of these patients that come in contact with these equipment are immunocompromised. Therefore, once they come in contact with these highly resistant pathogens as recorded in our study, these organisms can establish themselves on the body of patients and thus cause nosocomial infections. Thus, constant decontamination of hospital environment, equipment and fomites used in the treatment and housing of patients attending hospitals can help in preventing the spread of antibiotic resistant bacteria and preserve the efficacy of available antibiotics.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest

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