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Characterization and Susceptibility Pattern of Aerobic Bacterial Pathogens Isolated from Surgical Site Infection at Murtala Muhammad Specialist Hospital, Kano.Adamu, S. S.¹, Mohammed, Y. *¹, Ramadan, T.H. ¹, Takalmawa, U.H. ¹, Gadanya, M.A.², Sani, N.M.³

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Abstract

This study was a hospital- based cross- sectional descriptive study aimed at evaluating the characterization and susceptibility pattern of aerobic bacterial pathogens where clinical samples of wound swabs were aseptically collected from 364 patients with clinical evidence of post operative wound infections. Specimens were cultured for colony characteristics followed by direct Gram stain and biochemical tests for identification of pathogenic bacteria. Antibiotic susceptibility testing was done using a modified form of the Kirby Bauer method. Methicillin resistance *Staphylococcus aureus* (MRSA) was screened by Disc diffusion test using and phenotypic tests for screening extended spectrum beta lactamase (ESBL) on all gram-negative isolates using a double disk synergistic method. The mean age of the patients was 28.8 years, male was 210 (58%) and 154 (42%) female. Of the 364 swab samples, bacterial growth was obtained in 350 (96%) with positive culture and remaining 14 (4%) showed no growth. A total of 380 bacteria 345(90.79%) mono-isolate and 35(9.21%) mixed growth was isolated from the swabs with 300 (78.95%) gram negative bacilli. *E. coli* was the most predominant followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter* species, *Enterobacter* species, while 80(21.0%) were gram positive cocci. *Staphylococcus aureus* was more prevalent 64(16.48%) followed by Coagulase negative *Staphylococcus* (CoNS) with 15(3.94%) and the least was *Enterococcus faecalis* 1(0.25%). Majority of the gram-negative isolates showed multiple drugs resistance to most of the antibiotic tested but were highly effective to Imipenem. Extended Spectrum β -Lactamase (ESBL) production was seen in 17% but all were sensitive to carbapenems while thirty nine percent (39%) of the *S aureus* isolated were found to be multi-drug resistant *S aureus* (MRSA), and no clindamycin resistance was

obtained. The study demonstrated that most of gram negative isolates were multidrug resistant with an increase in ESBLs as well as MRSA strains.

Keywords: Antimicrobial, Susceptibility, Bacterial Pathogens, Surgical Site Infection, Kano

Introduction

Surgical site infection (SSI) account for 20% to 25% of all hospital acquired infections worldwide (Mawalla *et al.*, 2011). Hospital Acquired Infections (HAIs) are further complicated by an increasing prevalence of multidrug resistant organisms like methicillin resistant *Staphylococcus aureus* (MRSA), methicillin resistant coagulase negative staphylococci (MRCoNS), vancomycin resistant enterococci (VRE), multidrug resistance *Escherichia coli*, and *Acinetobacter* spp. (Mulu *et al.*, 2012; Kirkland *et al.*, 1999). It doubles the patient's risk of death after surgery (Kirkland *et al.*, 1999). These facts make SSI an important public health problem and therefore a target for improving the quality of care to the patient who are candidates for surgical treatment (Murray *et al.*, 2010). Lack of standardized criteria for diagnosis presents a challenge to monitor the global epidemiology of surgical site infection. The situation is serious in developing countries including Nigeria due to limited resources and irrational prescriptions of antimicrobial agent (Mawalla *et al.*, 2011). In addition to inadequacy and inappropriate use of preoperative Antimicrobial prophylaxis (AMP) is known to be a major risk factor associated with increased incidence of SSI (Saxer *et al.*, 2009).

Despite improvements in operating room practices, instrument sterilization methods, better surgical technique and the best efforts of infection prevention strategies, surgical site infections remain a major cause of hospital-acquired infections and rates are

increasing globally even in hospitals with standard modern facilities, protocols of preoperative preparation and antibiotic prophylaxis. In developing countries where resources are limited, even basic life-saving operations, such as appendectomies and cesarean sections, are associated with high infection rates and mortality (Abdel-aziz et al., 2013; Mawalla et al., 2011; Kirkland et al., 1999). The risk of acquiring hospital infection on hospitalized patients in relation to surgery is high, since about 77% of death of patients with hospital acquired infections was reported to be related with post operative infections (Amare et al., 2011). Furthermore, overcrowding and understaffing in hospitals results in inadequate infection control practices and lack of infection control policies, guidelines and trained professional also adds to the extent of the problem (Nejad et al., 2011).

Surgical site infection (SSI) can be reduced by appropriate use of surgical antimicrobial prophylaxis. In hospital practice 30- 50% of antibiotics are prescribed for surgical prophylaxis and 30-90% of this prophylaxis is inappropriate (Munckhof, 2005). This inappropriate use increases selection pressure favoring emergence of pathogenic drug resistant bacteria (Al-Momany et al., 2009). More ever, appropriate care and maintenance of operating rooms, including appropriate air handling and optimal cleaning and disinfection of equipment and the environment should be strictly adhered to. Also, surveillance of SSI with feedback of appropriate data to surgeons has been shown to be an important component of strategies to reduce SSI risk (Mangram et al., 1999).

Identifying the microbes associated with post SSI and determining their susceptibility pattern would provide better understanding of the spectrum of pathogens causing SSI as well as their susceptibility pattern which is important for prompt management of patients, as antimicrobial therapy significantly influences the outcome of the patients with SSI. Having such data would help to establish guidelines for the management of SSIs and contribute to planning of surveillance, prevention and control of SSI. The data also could be adopted by the policy makers to set a basic foundation for further extensive surveillance studies and add to existing data on the burden of SSI within the country. The aim of this study is to isolate and characterize the bacterial pathogens from surgical site infections and determine their susceptibility pattern at Murtala Muhammad Specialist Hospital (MMSH) in Kano State, Nigeria.

Materials and Methods

Study Design and Technique

This was a hospital based descriptive cross-sectional study conducted between November, 2016 to November, 2017. Non-probability, convenient sampling technique was employed for recruitment of all eligible patients.

Study Area

The sample collection was carried out in Department of Surgery of MMHS Kano-Nigeria, and all the laboratory analysis was done in the Department of Medical Microbiology and Parasitology, Aminu Kano Teaching Hospital, Kano.

Study Population

This study included all patients who showed clinical evidence of post operative wound infections (patients with complaints of discharge, pain, swelling, foul smelling and chronic wound) as diagnosed by the physicians.

Inclusion Criteria

Inclusion criteria included; patients of all age groups except neonates, presence of post-operative SSIs and giving verbal informed consent to participate in the study.

Exclusion Criteria

The following were excluded from this study; neonates, patients with infections occurring 30 days after operation if no implant is in place, infection following episiotomy and patients with burn injuries.

Sample size:

Three hundred and sixty four (364) samples were collected which was determined using the formula in a previous report (Lwanga and Lemeshow, 1991) based on local prevalence study done in Kano State with 30.1% (Emmanuel et al., 2014).

$$N = \frac{Z^2 \times P(1-P)}{d^2}$$

$$= \frac{1.96^2 \times 0.30(1-0.30)}{0.05^2}$$

Where N= sample size
 Z= standard normal variate (95% confidence level)=1.96
 P= incidence rate from past studies=30.1%
 d= absolute error = 5%
 N= 3446.7 + Attrition 5% of 347=17
 Therefore, the samples size used was =364.

Ethical Clearance

An ethical approval was obtained from the Ethical Review Board in Kano State Hospital Management Board. Structured questionnaires were used to extract data from the patients file.

Laboratory Procedures Specimen Collection

The specimens were collected aseptically on the first day when patients presented with clinical evidence of infection (purulent drainage from incision or drain) before the wound was cleaned with antiseptic. Using sterile swabs stick and obtained from surgical site without contaminating with skin commensals and transported to the laboratory immediately on Amies transport media.

Laboratory Analysis

Specimens were immediately cultured upon arrival in to the laboratory. Culturing for colony characteristics followed by direct Gram stain and biochemical tests were used to identify pathogenic bacteria. Culture media used were Blood (BA) and MacConkey (MCA) agar which was incubated aerobically on to the media at 37°C for 24-48hrs (CLSI, 2015).

Inoculation of Sample on to the Culture Media

The sample were aseptically inoculated on the primary media/plate MacConkey agar, Blood agar and SDA agar which is carried out by applying the swab bearing the sample on the surface of the agar to a size of 2cm square to produce the pool of the inoculums and then streaked out with a sterile wire loop flamed at interval of each streaking. To about 3-4 quadrant in order to obtained discrete colony. Then direct Gram staining was done before the storage of the swab stick. The culture on BA media was incubated in Co₂ enriched ATM candle jar to provide microaerophilic environment, while culture on MCA and SDA were incubated aerobically at 37 °C for a period of 24 hours. All these were done in accordance with the procedure explained by Cheesbrough (2010).

Identification of isolates

The characterization of bacterial isolates was based on **Gram staining, isolation and identification techniques**. These were conducted according to the method of Chessbrough (2010). Biochemical test was carried out on both the gram-negative isolate and gram-positive isolates as described by the Clinical Laboratory Standard Institute (CLSI, 2015).

Antimicrobial Susceptibility Testing of the Isolate

All isolated bacterial pathogens, following their

primary identification, were purified and subjected to antibiotic susceptibility testing using a modified form of the Kirby Bauer method (Arora and Arora, 2012; Bauer *et al.*, 1966). Ten commercially prepared discs oxiod for gram negative organism and eight commercially prepared discs oxiod for gram positive organism respectively were used in the study. Single disc antimicrobial agents (Oxoid, Basingstoke United Kingdom) of penicillin (10 units), ampicillin (10µg), amoxicillin/clavulanic acid (20/10µg), gentamycin (10µg), erythromycin (15µg), tetracycline (30µg), ciprofloxacin (5µg), clindamycin (2µg), triomethoprim/sulfamethaxazole (1.25/23.75µg) and chloramphenicol (30µg) were dispensed onto the surface of the agar plates. The plates were aerobically incubated at 37°C for 18 -24 hours. Results were recorded by measuring the diameter of the zone of inhibition around the antibiotic (CLSI, 2015).

Methicillin resistance *Staphylococcus aureus* (MRSA) Screening

Methicillin resistance *Staphylococcus aureus* (MRSA) was screened by Disc diffusion test using cefoxitin (30µg) disc on Mueller –Hinton Agar. Plates were incubated and maintained at 37°C for 24 hours. Results were interpreted according to standard guidelines (CLSI, 2015). Induced clindamycin resistance among *Staphylococcus aureus* was also screened by disc diffusion method on Mueller-Hinton Agar using 15µg of erythromycin disc and 2µg of clindamycin disc were spaced 15mm apart and incubated at 37°C for 18-24 hours. Flattening of the zone of inhibition adjacent to erythromycin (D-zone) was interpreted as inducible clindamycin resistance. Gram negative organisms were tested against (10µg), amoxicillin/clavulanic acid (20/10µg), ceftriaxone (30µg), ceftazidime (30µg), cefotaxime (30µg) gentamycin (10µg), tetracycline (30µg), ciprofloxacin (5µg), chloramphenicol (30µg) and imipenem (10µg).

Phenotypic Tests for Screening Extended Spectrum Beta Lactamase (ESBL)

All gram-negative isolates were screened for Extended Spectrum β-lactamase (ESBL). A double disk synergistic method using Amoxicillin clavulanic acid and two third generation Cephalosporins and Ceftazidime was adopted. A ≥ 5mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone is interpreted as ESBL positive (CLSI, 2015).

Statistical Analysis

The data generated in this study was analyzed using the Statistical Package for Social Sciences (SPSS) for windows version 20.0 used for statistical analysis and data interpretation. The values were expressed as means and percentages.

Results

A total number of 364 swab specimens from patients with clinical signs of post-operative SSIs were collected, processed and analyzed for the presence of aerobic bacterial pathogens. In this study, the mean age of the patients was 28.8 years, age range were 11-67 years, majority of the study population were male 210 (58%) and 154 (42%) were female (Table 1 and Figure 1).

Table 2 shows the distribution of Bacterial pathogens identified from these surgical site infections. Of the 364 swab specimens obtained, 350 (96%) yielded growth of bacteria with positive culture and remaining 14 (4%) yielded no growth (bacteriologically sterile). A total of 380 isolates were recovered from the 364 samples taken. The presence of only one species isolated from each sample (mono-isolate) was the most frequent with 345 (90.79%) while, more than one species was isolated (mixed growth) was 35(9.21%). Gram negative organisms accounted for 300 (78.95%) of the total number of organisms. Overall, *Escherichia coli* was the predominant organism isolated followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgris*, *Citrobacter specie*, *Enterobacter specie* accounted for (18.3%, 8.7%, 11.1% and 2.4%) of the total isolates respectively. Gram negative organisms accounted for 80(21.0%) of the total isolates. *Staphylococcus aureus* was more prevalent with 64(16.48%) followed by Coagulase negative *staphylococci* (*CoNS*) 15(3.94%) and the least was *Enterococcus faecalis* 1(0.25%).

Gram positive bacteria were tested against some selected commonly used antibiotics. The results obtained as shown in figure 3 indicated that the organisms varied in their susceptibility to all the antimicrobials used. Out of 64 *S. aureus* isolated obtained 25(39%) were found to be MRSA strain and no inducible clindamycin resistance was obtained (ICR), and among the remaining 39(61%), 37 (94%) showed high resistance rate to penicillin and tetracycline, 24(62%) and a moderate resistance to amoxicillin/clavulanic acid 21(54%), erythromycin 16(41%), chloramphenicol 28(37%) cotrimoxazole and relatively sensitive to gentamycin 24(59%) and

ciproflaxacin 37(62%) but highly sensitive to clindamycin. Coagulase negative *staphylococci* (*CoNS*) isolate shows low to moderate resistance to all the antibiotic tested but highly sensitive to clindamycin. *Enterococcus faecalis* was highly resistance to penicillin and Tetracycline but sensitive to most of the other classes of antibiotic tested. NRSA strains are highly resistance to most of the classes of antibiotic tested but very effective to clindamycin.

The resistance and susceptibility patterns of gram negative bacteria isolated from wound infections and tested against selected antimicrobial agents were shown in figure 4. Majority of them showed multi-resistances (resistance to two or more classes of antimicrobials) to most of the antibiotic tested but were highly effective to Imipenem. *Escherichia coli* showed high rate resistance to amoxicillin/clavulanic acid 64(66%), Ceftriazone, 76(78%) cefotaxime 64(66%) ceftazidime 56 (57%) but moderately sensitive to ciproflaxacin 67(69%) and gentamycin 66(68%) and highly effective to imipenem. *Klebsiella pneumoniae* showed high resistant rate the amoxicillin/clavulanic acid 34(85%) ceftriazone 38(96%), ceftazidime 32(80%), cefotaxime 32(80%) cotrimoxazole 18(45%) ciproflaxacin 28(70%) chloramphenicol 31(78%) but highly effective imipenem. *Pseudomonas aeruginosa* showed high resistant rate to amoxicillin/clavulanic acid 61(85%) ceftriazone 61(85%), cefotaxime 3(85%), ceftazidime 3(85%) and moderately sensitive to gentamycin 45(67%) and ciproflaxacin 46(42%) but 100% sensitive to imipenem which shows no resistance. While resistance rate of *Proteus mirabilis* to amoxicillin/Clavulanic acid was 25(83%) to chloramphenicol 23(77%), ceftriazone 26(87%), cefotaxime 26(87%), ceftazidime 25(83%) but showed low to moderate resistance to ciproflaxacin 18(60%), cotrimoxazole 20(67%), gentamycin 13(43%) 100% sensitive to imipenem. *Proteus vulgaris* showed low to moderate resistance to ciproflaxacin and gentamycin, high resistance to amoxicillin/Clavulanic acid 5(83%), ceftriazone 5(83%), ceftazidime 5(83%) cefotaxime 5(83%) chloramphenicol 4(67%) while 100% sensitive to imipenem. *Citrobacter specie* was found to be resistance to most of the antibiotic tested but highly effective against ciproflaxacin, chloramphenicol and 100% sensitive to imipenem.

Figure 4 shows the resistance pattern of ESBLs producing strains from this study. Out of the total 300-gram negative organism that were isolated from the clinical specimens, 53(17.7%) were ESBLs

producing strain. *Escherichia coli* account for 26(49%), *Klebsiella pneumoniae* 12 (22.6%), *Pseudomonas aeruginosa* 13 (25%) and *Proteus mirabilis* 2 (3.7%). This study shows that almost all these strains obtained were found to be highly

resistance to most of the classes of antibiotic tested, 100% resistance to 3rd generation cephalosporins but highly effective and sensitive to 4th generation cephalosporins, imipenem.

Table 1: Gender distribution of the study population

Gender	Frequency	Percentage (%)
Female	154	42
Male	210	58
Total	364	100

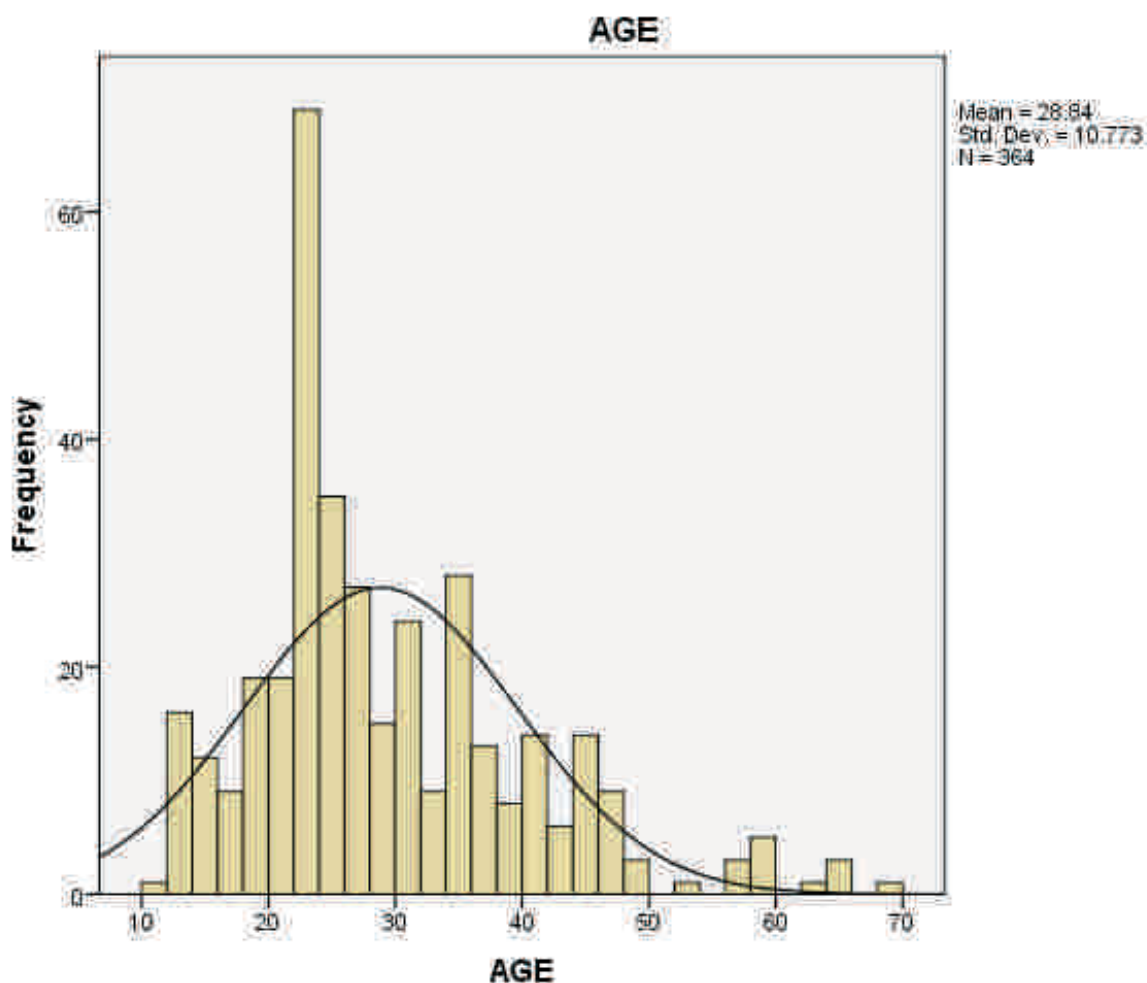


Figure 1: Age distribution of the study population

Table 2: Distribution of Pathogenic Bacterial Isolate From SSI

Bacteria	Frequency	Percentage (%)
<i>Staphylococcus aureus</i>	64	16.84
<i>Pseudomonas aeruginosa</i>	77	20.26
<i>Klebsiella pneumoniae</i>	52	13.64
<i>Citrobacter spp</i>	7	1.84
<i>Enterobacter spp</i>	3	0.79
<i>Proteus mirabilis</i>	32	8.42
<i>Proteusvulgris</i>	6	1.58
Coagulase negative staphylococci (CoNS)	15	3.95
<i>Escherichia coli</i>	123	32.37
<i>Enterococcus faecalis</i>	1	0.25
Total	380	100

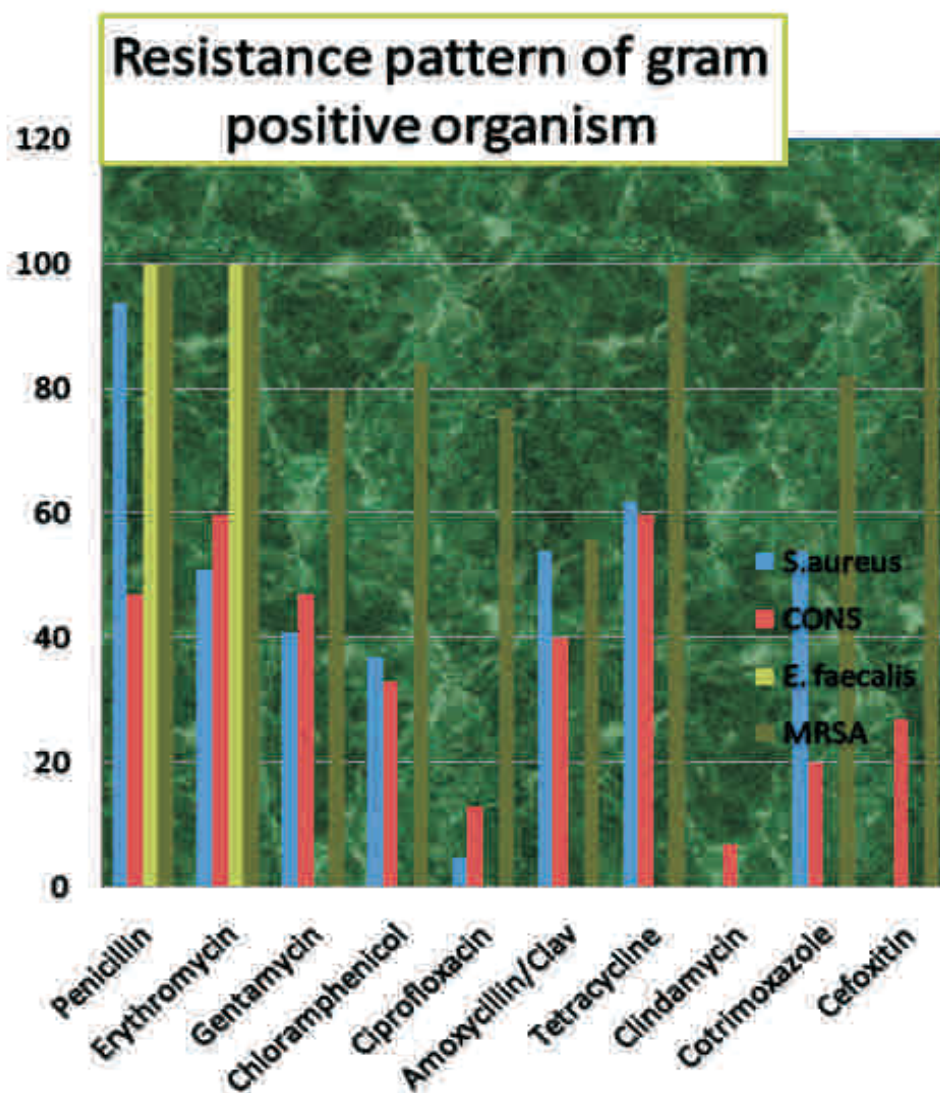


Figure 3: Antimicrobial resistance patterns of gram positive bacterial isolates

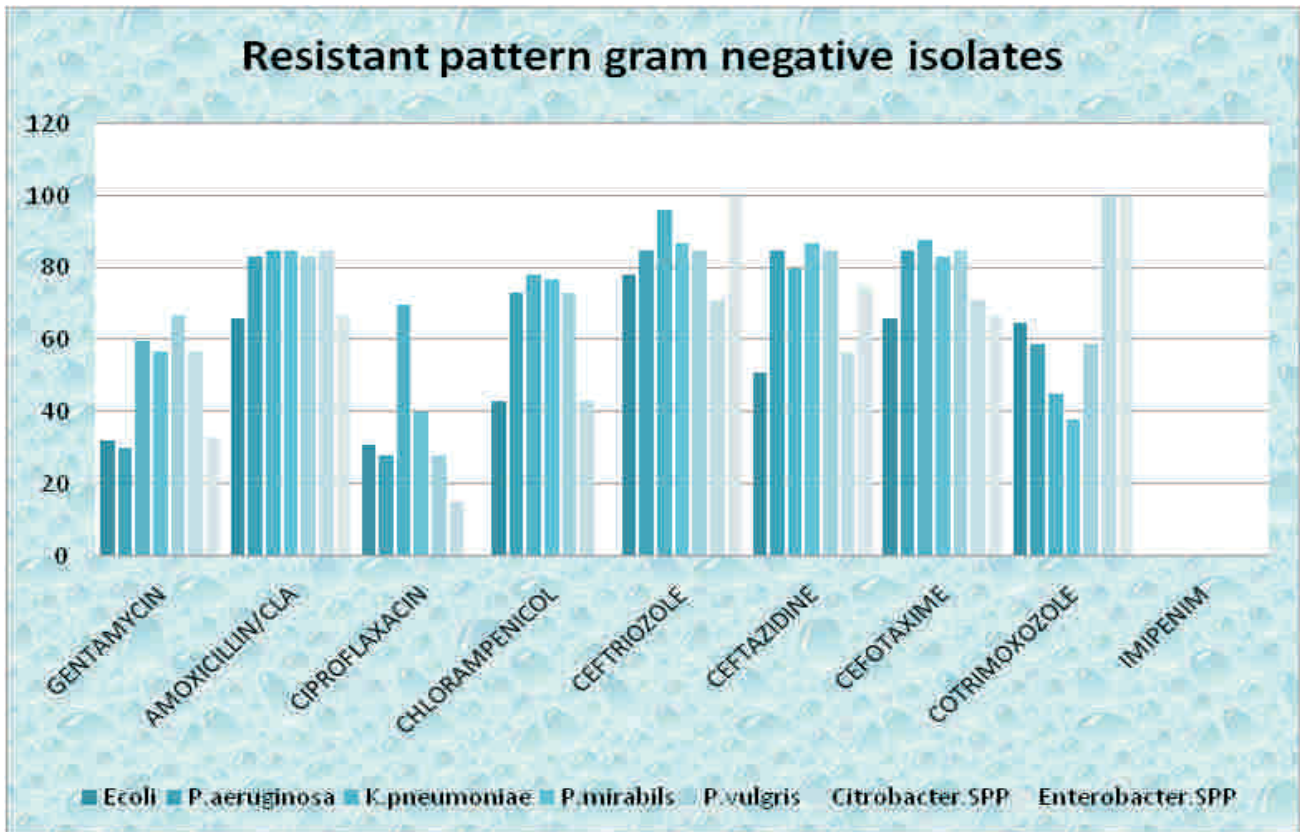


Figure 4: Antimicrobial resistance patterns of gram negative bacterial isolates

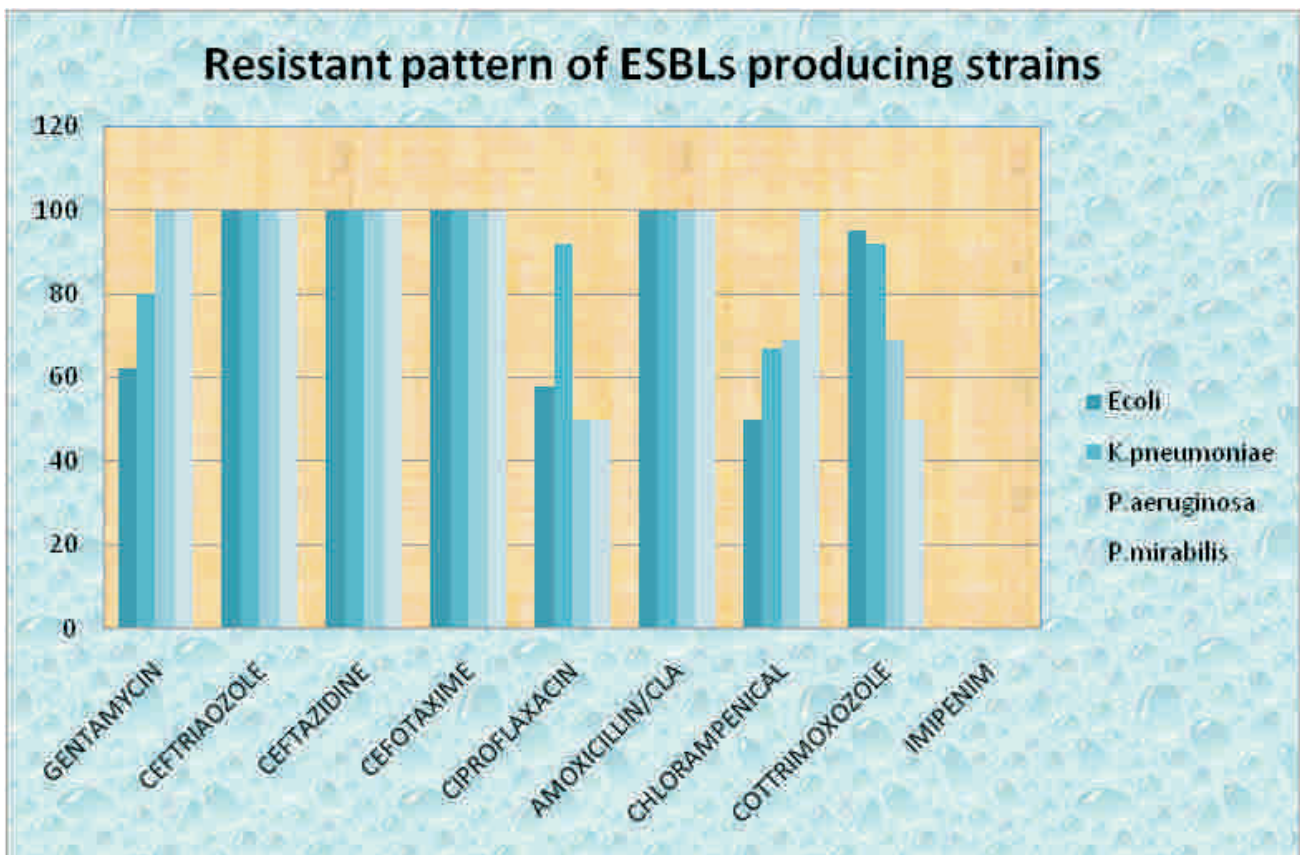


Figure 5: Antimicrobial resistance pattern of ESBLs-producing strains

Discussion

The present study gives an insight to the characteristics of causative pathogens of post operative wound infections and their susceptibility pattern. Wound infection was recorded more common in males (58 %) than in females (42%). This is in agreement with studies done in different parts of Nigeria by Amoran *et al.* (2013) who reported (51.3%) in males than (48.7%) in females in South Western Nigeria. Similarly, James *et al.* (2015) reported that males had the highest prevalence of 82.1% compared with the females who had 55.0% in a study done in North Central, Nigeria.

This study, documented that 90.79 % of culture positive wounds showed mono-microbial growth while 9.21% showed poly-microbial growth. The high percentage of mono-microbial growth in this study is particularly similar to those obtained in most parts of world; 91.6% in Ethiopia (Mohammedaman *et al.*, 2014), 86-100% in India (Suchitra and Lakshmidivi, 2009) and 98% in Pakiatan (Bibi *et al.*, 2012) respectively.

Our finding demonstrates the predominance of Gram negative bacterial isolates in SSIs, *Escherichia coli* being the commonest followed by *Pseudomonas aeruginosa*, *S. aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter spp* and *Enterobacter spp*. This pattern of organisms causing SSIs in the present study is in agreement with previous studies conducted in the same setting (Walelign *et al.*, 2016; Joel *et al.*, 2012). In contrast to other previous studies, that showed a higher proportion of Gram positive organisms, especially *S. aureus*, associated with SSI in different countries (Dessalegn *et al.*, 2014; Adegoke, *et al.*, 2010; Mahmoud *et al* 2000).

In this study gram negative bacteria displayed high rates of resistance to commonly prescribed antibiotics; gentamycin, chloramphenicol, sulphamethaxazole/trimethoprim, amoxicillin /clavulanic acid, ceftriaxone, cefotaxime, ceftazidime and tetracycline which is in agreement with different studies worldwide (Bibi *et al.*, 2012; Mulu, *et al.*, 2012; L.T.A. *et al.*, 2006; Amare, *et al.*, 2011; Mahmood, *et al.*, 2000).

This rate of resistance could be attributed due to the fact that, these antibiotics are easy to administer, relatively cheap and widely prescribed in empirical treatment of various bacterial infections in this study area. The high level of resistance to amoxicillin/ clavulanic acid in this study is relatively higher than previous data from the same study area reported as by Emmanuel *et al.* (2014) and another study conducted in Ethiopia by Walelign (2016). Resistance rate to sulphamethaxazole/trimethoprim (cotrimoxazole) in gram negative bacteria was recorded as 67% in this study is relatively lower compared to that obtained from a study done in Tanzania (Joel, 2012) who reported 80%. Gentamicin and ciprofloxacin were relatively effective antibiotics against Gram negative organisms associated with SSI which was in agreement with previous study conducted by Mengesha *et al.* (2014) and Dinda, *et al.* (2013).

Resistance to third generation cephalosporins in gram negative bacteria in the current study was very high. This observation was in line with previous study done from the same hospital and other countries (Lyamuya, *et al.* 2011; Blomberg, 2004). It is alarming to note that the resistance to third generation cephalosporin appears to be increased compared to the previous study from the same hospital and other places worldwide (Emmanuel, *et al.*, 2014; Joel, 2012; Moyo, *et al.*, 2010). A possible explanation for the high resistance could be due to presence of ESBLs production in these strains and increased inappropriate prescription of ceftriaxone for surgical antimicrobial prophylaxis in this hospital. Furthermore, in this study majority of patients received ceftriazone as prophylaxis for prevention of surgical site infections probably may have hindered detection of ceftriaxone susceptible gram-negative bacteria.

Extended Spectrum Beta Lactamase (**ESBLs**) phenotype was found in 7.1%, 3.6%, 3.2%, and 0.5% of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis* isolates respectively in this study with an overall prevalence rate of 14.5%. Similarly, a lower prevalence rate was reported previously (Yushau, *et al.*, 2011) in screening of ESBLs among isolates of enterobacteriaceae in Aminu Kano Teaching

Hospital who documented a prevalence of 10.25%. This finding demonstrates an increasing of these of Extended Spectrum Beta Lactamase (ESBLs) strains in Nigeria. This study also found that majority of *S. aureus* isolates were highly resistant to penicillin and tetracycline. This finding is in agreement with previous studies done in Nigeria and other developing countries which reported high resistance rates of *S. aureus* to penicillin and tetracycline (Walelign, 2016; Bibi, *et al.*, 2012). *Staphylococcus aureus* and *CoNS* displayed moderate rate of resistance to amoxicillin-clavulanic acid, but highly effective to clindamycin, this finding is in line with previous study from other places elsewhere (Joel, 2012). Resistance to erythromycin was observed in only 51% of *S. aureus* and 60% *CoNS* isolates, the observation which was higher than previous data reported from Addis Ababa. Gentamycin resistance in *S. aureus* varies worldwide. Moderate resistance was noted in this study, which was lower than that reported from Pakistan (Rakhshanda *et al.*, 2014). The present study also documented a high rate of methicillin resistance among *S. aureus*; 39% of *S. aureus* obtained from this study were MRSA. This finding was relatively higher than what was reported 4 years ago from a study at the same hospital with 19.6% (Minjibir, 2013).

Conclusion and Recommendations

In conclusion, findings from our study have revealed the occurrence of multiple drug resistance Gram negative organisms with *E. coli* as the most predominant as well as increase in SSIs due to ESBLs producing strains and MRSA. This study also provided baseline information in assisting surgeons, physicians and clinical microbiologists on rational antimicrobial use and continuing surveillance of bacterial antimicrobial sensitivity tests at local level which will help to reduce emergence and spread of resistant bacterial isolates. Routine culture should be performed whenever SSIs is suspected. The use antimicrobial sensitivity (including MRSA and ESBL screening) test results to guide choice of antibiotics and the practice of aseptic technique during and after surgery should be strictly adhered to rather than overreliance on antibiotics to reduce emergence and spread of resistant pathogens. There is also the need to establish continuous surveillance

to monitor antimicrobial susceptibility pattern of the common isolates found in SSI and continuous dialogue between the microbiology unit and the surgeons in management of surgical wound infections.

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