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Antibiotic resistance and plasmid profile of bacteria associated with nosocomial infections: A case study of Ekiti -State Teaching Hospital

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Abstract

Many serious health problems resulting in an enormous burden of morbidity and high health care costs are traceable to infections caused by the nosocomial pathogens; major victims are relatives of patients, the care givers who are doing their legal duties and at times the patients whose conditions could be complicated. The causative organisms of these infections and their sensitivity to antibiotics need to be ascertained. This study investigated the antibiotic sensitivity and plasmid profile of bacteria associated with nosocomial infections. A total of 45 samples were collected from children, male and female surgical wards in Ekiti State Teaching Hospital, Ado-Ekiti by using swab sticks and settling plates method. Isolation of bacteria, antibiotic sensitivity and plasmid profiling of the isolates were carried out using standard microbiological methods. The results showed that a total of 56 bacterial isolates were obtained from the 3 different wards, this includes *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, *Lactobacillus fermenti, Bacillus subtilis, Bacillus cereus* and *Micrococcus luteus. Staphylococcus aureus* had the highest frequency of occurrence with 30(53.6%), followed by *Staphylococcus epidermidis* 10(17.7%), *Escherichia coli* 7(12.5%), *Lactobacillus fermentii* and *Micrococcus luteus* 3(5.4%) and *Bacillus subtilis* 2(3.6%) respectively, while *Bacillus cereus* 1(1.8%) had the least frequency of occurrence. The gram positive isolates were more resistant to the antibiotics compare to the gram negative isolates, *Staphylococcus aureus* was the most resistant of all the isolates followed by *Staphylococcus epidermidis,* the five representative resistant isolates harbored plasmid of different sizes. The findings of this study suggests that patients, care givers and visitors to the hospital should maintain good personal hygiene and proper sanitary measures to avoid nosocomial infections, therefore, surveillance of nosocomial pathogens is important to reduce hospital stay, cost, morbidity and mortality rate.

Keywords: Antibiotics sensitivity; Bacteria; Hospital; Nosocomial infections; Patients and Plasmid profile

1. Introduction

Nosocomial infections, otherwise known as hospital-acquired infections are the infections acquired in the hospitals or healthcare service units such as the nursing home, rehabilitation facilities and outpatient clinics, these infections were not present during the time of admission (Anna and Farah, 2023). These infections result to serious health problems with an enormous burden of morbidity and high health care costs. They first appear 48 hours or more after hospital admission or within 30 days after discharge following in patient care (Dantas *et al.,* 2014). They are unrelated to the original illness that brings patients to the hospital and neither present nor incubating as at the time of admission (Li *et al.,* 2016). It is sometimes called a health care associated infections (HAI or HCAI) and it may range from mild to severe (Sheikh *et al*., 2023). The infections are spread to the susceptible patients in the clinical setting by various means, it may originate from health care staff, contaminated equipment, bed linens, or air droplets. The infection can also originate from the outside environment, from another infected patients or other sources which cannot be determined ((Jennifer,

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2021)). In some cases, the microorganisms may originate from the patient's own skin microbiota becoming opportunistic after surgery or other procedures that compromise the protective skin barrier (Doyle *et al.,* 2011).

The most common types of nosocomial infections are bloodstream infections (BSI), pneumonia (ventilator-associated pneumonia [VAP]), respiratory infections, urinary tract infection (UTI), gastroenteritis, meningitis and surgical site infections (SSI) (Raka *et al.,* 2006, Itani *et al.,* 2011). The bacteria that cause nosocomial infections include *Staphylococcus aureus*, *Streptococcus* spp., *Bacillus cereus, Acinetobacter spp.,* coagulase negative *staphylococci, enterococci, Pseudomonas aeruginosa, Legionella* and members of the Enterobacteriaceae family such as *Escherichia coli*, *Proteus mirabilis, Salmonella spp*., *Serratia marcescens* and *Klebsiella pneumonia* (Peter *et al*., 2017). But the most frequently reported nosocomial pathogens have been *E. coli, S. aureus, enterococci* and *P. aeruginosa* (Teresa *et al.,* 2008). Risk factors for the development of nosocomial infections include poor nutritional status, exposure to multiple antibiotics, indwelling central venous catheters; mechanical ventilation, poor hygiene practice by the patients and the care givers and length of stay in the hospital (Doyle *et al.,* 2011).

The prevalence rate of nosocomial infections as studied by WHO is averagely 11.85% (Kaye *et al*., 2014), the risks are increasing in developing nations annually because it has been estimated that between 5 and 10% of patients admitted to the hospitals for care acquired one or more infections. Over 1.4 million people worldwide suffer from infection complications acquired in hospital, the highest frequencies of nosocomial infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8 and 10.0%, respectively), and a prevalence of 7.7 and 9.0% in the European and Western Pacific Regions respectively ((Li *et al.,* 2016). 25 to 50% of nosocomial infections are due to the combined effect of the patients own flora and invasive devices. Nosocomial infections have posed a problem of enormous magnitude globally, because hospital localities have proven favourable in transmitting infections due to existing suitable pathogen-host- environment relationship. Estimates from various countries show that at any point in time, a significant number of hospitalized patients develop infections which were not present or incubating when the patients were admitted to hospitals (Urrea *et al*., 2014). Public and hospital staff awareness of nosocomial infections is either very low or none with many healthcare institutions having no policy on disease management and guidelines on combating nosocomial infections.

Nosocomial infections are also important public health problems in developing countries, as well as in developed countries (Jennifer, 2021). The socioeconomic impact for instance, prolongation of hospitalization, mortality, and cost of these infections adversely affects patients and nations economic wellbeing (Itani et al., 2014). Nosocomial infections pose a threat to the patients especially in the high-risk departments, such as the Intensive Care Unit (ICU) because it may result in an excess length of stay in hospital. It also reduces the chances of treatment for others and emergence of multiple antibiotic resistance microorganisms (WHO, 2012).

Infections cause by the nosocomial pathogens are the leading causes of death worldwide, major victims are relatives of patients, the care givers and at times the patients whose conditions could be complicated, they are also serious health problems resulting in an enormous burden of morbidity, mortality rates and high health care costs, this may be due to the resistance of nosocomial pathogens to drugs (Sheikh *et al*., 2023). It has been reported that more than 70% of these pathogens from the hospital environments are resistant to drugs (Grohskopf *et al.,* 2012), Nosocomial infections pose a threat to the patients due to the occurrence of multi-drug resistance in hospital-associated pathogens which has resulted in the emergence and re-emergence of difficult - to – treat nosocomial infections in patients (Doyle *et al.,* 2011). Therefore, the causative organisms of these infections, their sensitivity to antibiotics and the plasmid profile need to be ascertained. This study investigated the antibiotic sensitivity and plasmid profile of bacteria associated with nosocomial infections

2. Materials and methods

2.1. Collection of samples

Samples such as (swab from walls, beds, chairs, sinks) were collected from the hospital and some of the plates were exposed to the environment using setting plate method as described by Horan *et al.* (2008). The samples were labelled, packed and transported to the Laboratory within 30 minutes of collection.

2.2. Isolation of bacteria

The media used (Nutrient agar, Eosin methylene blue agar, Mannitol Salt agar) were prepared according to manufacturer's instruction, each medium was poured into separate petri dish and allowed to solidify. Swab of each

sample was streaked on the appropriate medium and incubated at 37^{0}C for 24hours and the exposed plates were also incubated at 37 ^{o}C for 24 hours. All the plates were then observed for the presence of bacterial growth.

2.3. Identification of bacterial isolates

The bacterial isolates were identified on the basis of cultural, morphological, and biochemical tests such as sugar fermentation, coagulase test, catalase and spore staining (Olutiola *et al.,* 2000). In order to determine the identity of bacteria isolates, the results were compared with standard references of Bergey's Manual of Determinative Bacteriology 2nd edition.

2.4. Antibiotic sensitivity testing

Antibiotic sensitivity test was carried out according to Clinical and Laboratory Standards Institute (CLSI, 2005). Each isolate was inoculated on sterile Muller Hinton agar plate, Gram-positive or Gram-negative standard antibiotic discs was placed on each plate, all the plates were incubated for 24hours at 37^{0}C. The plates were examined for the presence of zone of inhibition.

2.5. Plasmid Analysis

Plasmid extraction was carried out based on the methods of Molina *et al*. (2009) with little modification. Five milliliter of overnight broth culture of each of the five (5) representative resistant isolates was centrifuged in an Eppendorf tube at 10,000 rpm for 2 minutes. The supernatant (Liquid) was decanted, a micropipette was used to drawn off all the liquid that remains in the tube and 100ml of TET buffer was added.

The cells were suspended by closing the tube, two hundred ml (200) of SDS/ NAOH solution was added, mixed gently by inverting the closed micro centrifuge tube and left for 5 minutes at room temperature. One hundred and fifty (150) mL of KOAC was added and left on ice for five minutes. The tube was then centrifuged for five minutes to spin down the cell debris and chromosomal DNA. Four hundred (400) mL of the supernatant (Liquid) was transferred carefully into a clean micro centrifuge tube, it was ensured that none of the cells debris was carried over. The supernatant contains the plasmid DNA, 400 mL of ice cold ethanol was added, gently mixed and left in a deep freezer (-18 to – 20 °C) for 10-30 minutes, and then centrifuged for ten minutes at high speed. The supernatant was decanted into a waste container taking care so that the pellet of plasmid DNA was not discarded. The last drops of the liquid was removed with a micropipette, the pellet was dissolved in fifteen mL of TE buffer, mixed vigorously and stored in a freezer.

2 g of 2% agarose gel was microwaved for 1 – 3 minutes until it has been completely dissolved. The agarose solution was allowed to cool down to about 50 ^{o}C, Ethidium bromide (EtBr) was added to a final concentration of approximately 0.2 – 0.5 µg/m, EtBr binds to the DNA and allows to visualize the DNA under ultraviolet (UV) light. The agarose was poured into a gel tray with the well comb in place, a molecular weight ladder was carefully loaded into the first lane of the gel, loaded dye was added to each of the samples and it was loaded into each well. The agarose gel was placed into the gel box (electrophoresis unit) which was filled with TBE buffer until the gel is covered and all of the EtBr were on the top portion and the bands were differentially intense. The gel was run at 120 V until the dye line is approximately 75-80% of the way down the gel.

2.6. Curing of the resistant isolates

Curing of the resistant isolates was carried out by exposing the overnight grown culture of each representative resistant isolate to an elevated temperature of 37 ^{o}C and 10 mg/mL of ethidium bromide according to the method of Zama *et al*. (2010) in order to remove their antibiotics resistance ability.

2.7. Antibiotic Sensitivity of the cured bacterial isolates.

The antibiotics sensitivity of the cured isolates was carried out using disc diffusion method according CLSI, 2005 by inoculating broth cultures of each cured isolate on sterilized Muller Hinton agar plates. Both gram positive and gram negative antibiotics disks were placed separately on the inoculated plates and incubated for 24 hours, the plates were observed for the presence of zone of inhibition.

3. Results and Discussion

Tables 1,2 and 3 show the bacteria isolated from different wards, a total of 56 bacterial isolates belonging to 7 genera were isolated from the wards (children, male and female surgical ward), these includes: *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Lactobacillus fermenti, Bacillus subtilis, Micrococcus luteus* and *Bacillus cereus*. The presence of these isolates in the hospital environments implies contamination and people are susceptible to contacting different pathogens which can lead to disease conditions such as boils, impertigo, carbuncle, furuncle, nausea, fever, food poisoning and dysentery.

The high prevalence of bacterial isolates from the hospital environments may be due to poor hygienic practice in the wards, shortage of water in the hospital, uncared attitude of the patients, care givers and the visitors, patients may sometimes fail or forget to wash their hands after visiting the toilet or bathroom and thereby transfer the bacteria to the wall, bed and chair in the hospital or to the mouth during meals. This work is in line with the work of Theresa *et al.* (2008) who reported that the most frequently reported nosocomial pathogens were *E.coli, S. aureus* and *Enterococci*. The presence of these organisms in the hospital may be due to lack of basic facilities for maintaining standard hygienic conditions which is common in African countries including Nigeria (Fontana *et al*., 2000).

Table 1 Bacterial Isolates from Children ward

Locations	Isolates
W1	*Staphylococcus aureus*
W2	*S. aureus*
W3	*S. aureus, Staphylococcus epidermidis*
Ch1	*S. aureus*
Ch2	*Escherichia coli*, *S. aureus*
Ch3	*Micrococcus luteus*
Bd1	*S. aureus*
Bd2	*S. aureus*
Bd3	*Escherichia coli, S. epidermidis*
Sk1	*Staph. Epidermidis*
Sk2	*S. aureus, E. coli*
Sk3	*S. aureus, S. epidermidis*
Ep1	*S. aureus, S. epidermidis*
Ep2	*S. aureus, E. coli*
Ep3	*S. aureus*

Key: W = wall, Ch = chair, Bd = Bed, Sk = sink, Ep = exposed plates

Table 2 Bacterial isolates from the Male surgical ward

Locations	Isolates
W1	*Micrococcus luteus*
W2	*Bacillus subtilis*
W3	*S. aureus, Staphyloccus. epidermidis*
Ch1	*Bacillus cereus*
Ch2	*S. epidermidis*

Ch3	*M. luteus*
Bd1	*B. subtilis*
Bd2	*S. aureus*
Bd3	*S. aureus*
Sk1	*S. aureus*
Sk2	*Escherichia coli*
Sk3	*S. aureus, S. epidermidis*
Ep1	*S. epidermidis, S. aureus*
Ep2	*S. aureus*
Ep3	*S. aureus*

Key: W = wall, Ch = chair, Bd = Bed, Sk = sink, Ep = exposed plates

Table 3 Bacterial isolates from the female surgical wards

Locations	**Isolates**
W1	*Staphylococcus. aureus*
W2	*Escherichia coli*
W3	*Staphylococcus. Epidermidis*
Ch1	*S. aureus*
Ch2	*Lactobacillus fermentii*
Ch3	*S. epidermidis*
Bd1	*S. aureus*
Bd2	*S. aureus*
Bd3	*Escherichia coli*
Sk1	*S. aureus*
Sk2	*S. aureus*
Sk3	*S. aureus*
Ep1	*Lactobacillus fermentii, S. aureus*
Ep2	*Lactobacillus fermentii*
Ep3	*S. aureus*

Key: W = wall, Ch = chair, Bd = Bed, Sk = sink, Ep = exposed plates

Figure 1 showed the frequency of occurrence of the bacterial isolates from the wards, *Staphylococcus aureus* had the highest occurrence with 30(53.6%), this is followed by *Staphylococcus epidermidis* 10(17.7%), *Escherichia coli* 7(12.5%), *Lactobacillus fermenti* and *Micrococcus luteus* with 3(5.4%) and *Bacillus subtilis with* 2 (3.6), while *Bacillus cereus* had the lowest frequency of occurrence. *Staphylococcus aureus* was the most prevalence isolates because it is common everywhere and it is the normal flora of the body but may also be an opportunistic pathogen, Oni *et al.* (2006). In the work of Nejad *et al*. (2011), *Staphylococcus aureus* have been mostly recovered from post-operative surgical wards despite the site of infection and location of specimens due to its high survival characteristics in hospital environment (Dalhatu *et al*. 2014). It is known to rank second among nosocomial pathogens isolated from hospitals, often contaminating hospital equipment's such as sinks, wall, beds and other surgical apparatus; and even antibiotic resistant strains can survive in supposedly sterile equipment used in the hospitals, making it a dangerous nosocomial

pathogen widely distributed in the hospital environments where they are particularly difficult to eradicate (Masaadeh and Jaran 2009).

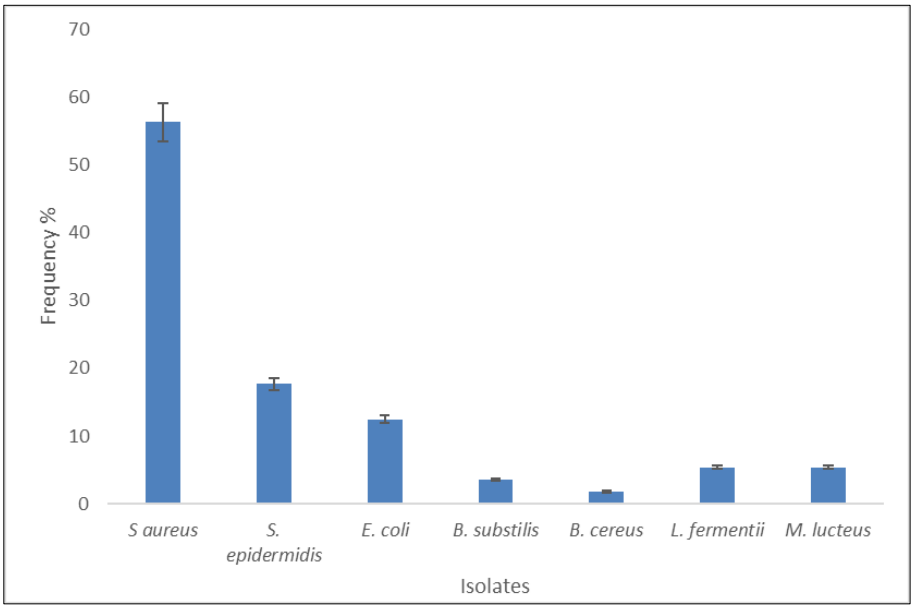
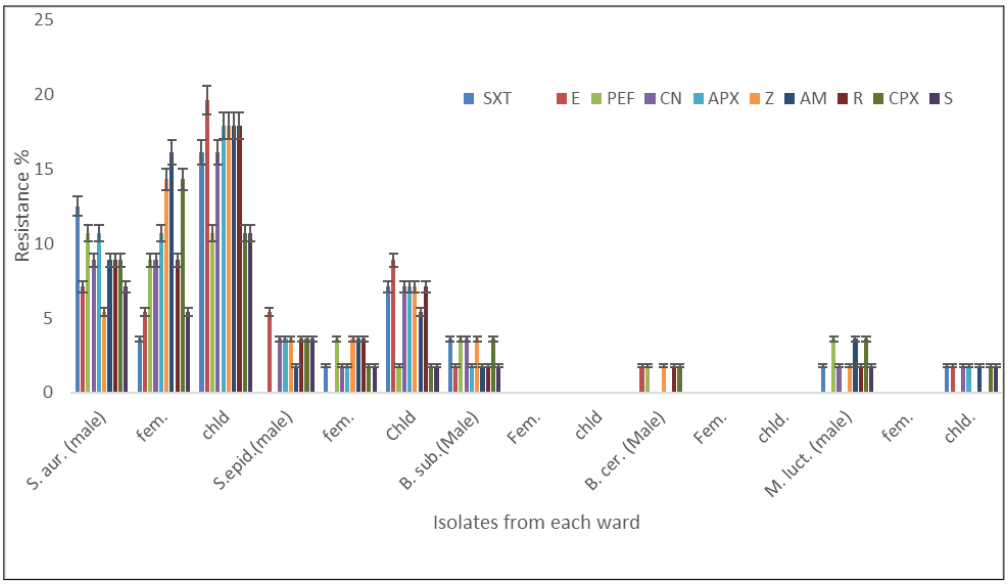


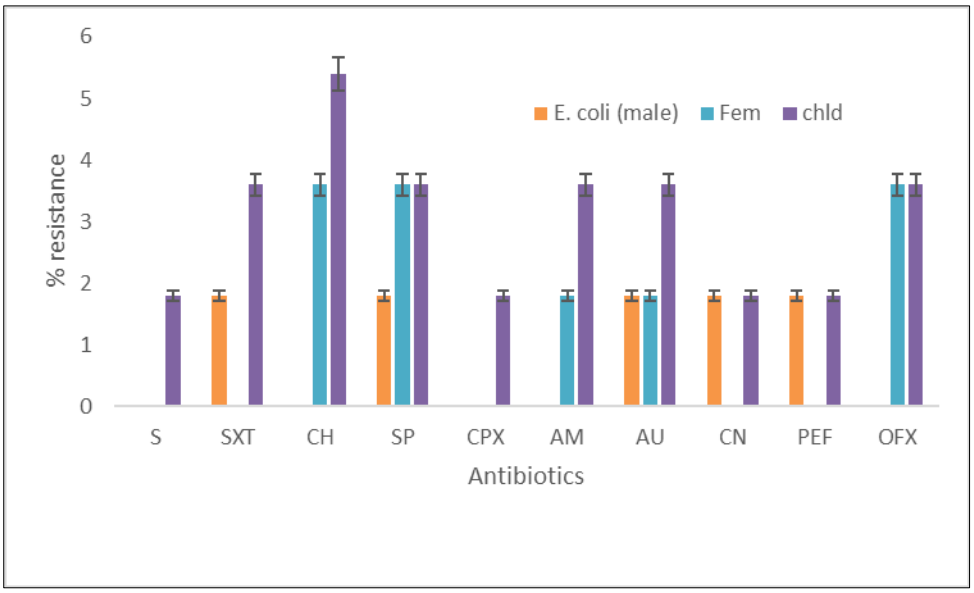
Figure 1 Frequency of occurrence of the bacterial isolates

Antibiotic sensitivity of the isolates were observed in figure 2 and 3, the isolates showed resistant to the antibiotics; *Staphylococcus aureus* was the most resistant of all the isolates followed by *Staphylococcus epidermidis, Lactobacillus fermentii, Escherichia coli, bacillus subtilis and Micrococcus luteus.* Oleghe *et al*. presumed that the acquisition of the resistance may be due to chromosomal mutations or through plasmid that are capable of transfer from one strain of organism to another even across the species in addition to environmental influence. The high level of antibiotic resistance among the isolates implies the presence of antibiotics resistance bacteria in the environment and resistance may be transferred to people from generation to generations; the resistance may also be due to inability of the antibiotics to penetrate the cell wall of the bacteria, mutation and possession of efflux pump by the bacteria. This result is in line with (Raymond and Aujard, 2010), where the authors observed antibiotics resistance in some bacteria.



Key: S.aur.= Staphylococcus. aureus, S. epid. = Staphylococcus. epidermidis, B. sub. = Bacillus subtilis, B. cer. = Bacillus cereus, M. luct. =Micrococcus luteus, fem= female, chld= children; SXT: Septin, E: Erythromycin, PEF: pefloxacin, CN: Gentamycin, APX: Ampiclox, Z: Zinnacef, AM: Amoxil, R: Rifampicin, CPX: Ciprofloxacin, S: Streptomycin.

Figure 2 Antibiotics resistance of Gram positive Isolates



Key: S: Streptomycin, SXT: Septrin, CH: Chloranphenicol, SP: Sparfloxacin CPX: Ciproflox, AM: Amoxil, AU: ugumentin, CN: Gentamycin, PEF: Reflacine, OFX: Tarifid

Figure 3 Antibiotics resistance of Gram negative isolates

All the 5 representative resistant isolates, *Staphylococcus. aureus (*female ward*), S. aureus (*exposed plate in male ward*), S. aureus (*from sink in male ward*), Staphylococcus. epidermidis* (children ward*) and Escherichia coli (*children ward*)* harbored plasmid. This showed that their resistance to antibiotics were mediated by plasmids, Mbim *et al*. (2016). The results of plasmid curing and antibiotic sensitivity test after curing showed that curing of plasmids was effective in all the isolates. This is an indication that the isolates have been cured of their plasmids, and therefore the resistance was plasmid mediated.

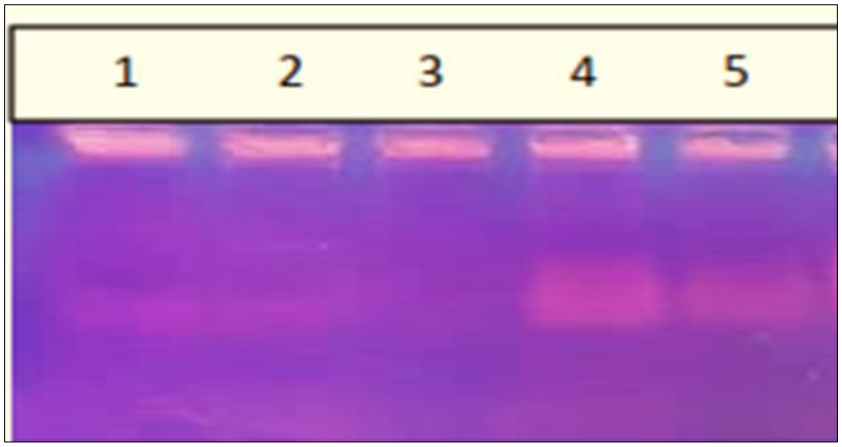


Plate 1 Plasmid profile of the bacterial isolates

Isolate 1: *Staphylococcus epidemidis* (from children ward), Isolate 2: *Staphylococcus aureus* (female ward), Isolate 3: *Staphylococcus aureus* (exposed plate in male ward), Isolate 4: *Staphylococcus aureus* (from sink in male ward), Isolate 5: *Escherichia coli* (children ward).

4. Conclusion and recommendation

Common bacteria found in the hospital environment were *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Lactobacillus fermenti, Bacillus subtilis, Micrococcus luteus* and *Bacillus cereus.* A high level of antibiotic resistance was observed among the bacterial isolates, although some of the isolates were susceptible to the antibiotics. The presence of plasmid in some isolates and their abilities to be cured of the plasmids showed that their resistance is plasmid mediated.

The findings of this study suggests that patients, care givers and visitors to the hospital should maintain good personal hygiene and proper sanitary measures to avoid nosocomial infections, therefore, surveillance of nosocomial pathogens is important to reduce hospital stay, cost, morbidity and mortality rate.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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