Original Research Article

ANTIBIOGRAM OF BACTERIA ISOLATED FROM POST - OPERATIVE WOUNDS OF MOTHERS WHO UNDERWENT CAESAREAN SECTION AT THE MOTHER AND CHILD HOSPITAL, AKURE, ONDO STATE.

ABSTRACT

This study was designed to determine the antibiogram of bacteria isolated from post - operative wound samples of mothers who underwent caesarean section at the Mother and Child Hospital, Akure. The collected samples were subjected to microbiological analysis and bacterial isolates were identified using conventional identification techniques. The antibiotic sensitivity profile of the bacterial isolates to commercially available antibiotics was determined using disc diffusion technique. Results obtained showed that *Staphylococcus aureus*, *Pseudomonas aeruginosa Proteus* sp and *E. coli* are the most frequently isolated strains from post - operative wound samples analysed. Moreover, most of the isolates displayed multi drug resistance to the conventional antibiotics used. This study has shown that multi drug resistant pathogenic organisms are predominant in post-operative caesarean wounds amongst the patients sampled. The implication of this is the tendency of such wounds to become septic and life threatening to the patients. There is therefore the need for the development of more effective chemotherapeutic drugs.

Keyword: Antibiogram, post-operative wound, caesarean section, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

1.0 INTRODUCTION

A wound is a breach in the skin that can lead to loss of skin integrity. It exposes the subcutaneous tissue as a result of cuts, scrapes, scratches or punctures which happens accidentally, during surgery (such as caesarean section), sutures or stitches, which creates easy entry of microorganisms leading to their proliferation (Valerie, 2016). Wound infection is regarded as the most common nosocomial infection especially in patients undergoing surgery. Caesarean section (CS) is the most common obstetric surgeries done in women of reproductive age group. Post-caesarean wound infection is a disturbing occurrence in spite all the techniques and measures to ensure aseptic condition. This infection has led to prolonged hospital stay, high hospital bills, as well as other morbidities and mortality (Agboeze *et al.*, 2014). Bacteria such as *Staphylococcus aureus*, *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa* are the most associated bacterial isolates (Church *et al.*, 2006). S. *aureus* was reported to have 72% of cases of Post-operative Wound infection (POWI) in implants while *E. coli* and *Klebsiella* species accounted for 14% each as reported in Lagos, Nigeria. In Jos, North Central Nigeria, the picture was slightly different where it was found that *Proteus* species were in 41.9% of wounds samples cultured while *S. aureus* was 25.6%. Coliforms (13.9%), S*treptococcus* Spp., *Pseudomonas* spp. and *Klebsiella* were the other isolates (Akinjogunla *et al.*, 2009). Minimizing the incidence of postoperative wound infection relies on adequate asepsis and antisepsis and preservation of the local host defenses (Bowler *et al.*, 2001).

Post-operative wound infection otherwise known as 'Surgical Site Infection' (SSI) (CDC, 1992; CDC, 1997) has proven to be a serious hazard to patients, with incidence according to CDC (1997) to be 15.5%, according to the UK nosocomial infection surveillance (2009) to be 11.32%, and according to ASEPSIS (2009) to be 8.79% as documented by (Ashby *et al.*, 2010). About 77% of the deaths of surgical patients are related to surgical wound infection (Mangram *et al.*, 1999). Surgical Site Infections are classified into incisional SSIs, which can be superficial or deep, or organ/space SSIs (Ashby *et al.*, 2010). The control of wound infections from CS has become more challenging due to widespread bacterial resistance to antibiotic and to a greater incidence of infections caused by methicillin-resistant *S. aureus*, polymicrobic flora and by fungi (Shittu *et al.*, 2002). The emergence of resistant strains of *S. aureus* has increased the morbidity and mortality associated with wound infections. Although, Vancomycin has been shown to be effective against Methicillin Resistant *S. aureus* (MRSA), however, some strains of *S. aureus* have been shown to be resistant to this vancomycin (Hemanth *et al*., 2004).

Moreover, bacteria such as *P aeruginosa, Klebsiella* sp and *Proteus* sp among others have also developed resistance to almost all known antibiotic. Actually, the spread of antimicrobial resistance is a global problem due to significant changes in microbial genetic ecology and as a result of indiscriminate use of antimicrobial agents. As a result, research efforts are now geared at the development of new agents to treat bacterial infections (Zhanel *et al*., 2006). There have been series of reports of wound infections in many hospitals around the globe, however, not much work has been done on antibiogram of post-CS wound infections in the community sampled in this investigation, that is, Akure, Nigeria. Therefore, this study was aimed at determining the microbiological pattern of post-CS wound infections in Akure town using the Mother and Child Hospital, Akure as case study and also to evaluate the antibiogram profile in order to reduce post-operative wound infections and associated morbidity and mortality (in severe cases).

2.0 **RESEARCH METHODOLOGY**

2.1. Collection of clinical samples

A total number of 35 wound swab samples were collected aseptically from Mother and Child Hospital, Akure, Ondo state, Nigeria and were transported to the laboratory of Microbiology at Federal University of Technology, Akure.

2.2 Ethical clearance/ Informed consent of patients

The certificate of ethical clearance was issued by the management of the hospital and the consent forms were filled by all the patients examined.

2.3. Isolation and identification of bacteria from wound infections.

The wound swabs collected were inoculated on a Mannitol Salt Agar (MSA), Eosin methylene blue agar, Nutrient agar (NA), Cystein Lactose Electrolyte Deficient Agar (CLED) using streaking method and was incubated aerobically at 37^{o}C for 24hours in an IPF400 Precision incubator (Memmert, Germany). The different bacteria colonies were identified on the basis of their morphological and biochemical characteristics as described by Cheesbrough (2006).

2.4. Morphological and biochemical characterization of isolated bacteria

The isolates were characterized morphologically first on agar plate and then by Gram's staining as described by Olutiola *et al.* (1991). A smear was made on a clean labeled slide using a sterile wire loop and then heat fixed. The smear was then flooded with crystal violet for 1 minute and rinsed off with slow flowing tap water; lugols' iodine solution was then added on the smear and allowed to react for 1 minute and then rinsed with slow flowing tap water. The smear was then decolorized with ethanol for 30 seconds and immediately rinsed off under gently running tap water so as to remove the alcohol effect. The slide containing the smear was then counterstained with safranin for 1 minute and rinsed with water. The slide was blotted dry. The presence of Gram-negative bacteria appeared pink while Gram-positive appeared purple when viewed under the oil-immersion microscope. For Biochemical characterization, the following tests were carried out on each of the isolates; citrate, catalase, coagulase, Triple sugar iron (TSI) test, sugar fermentation

2.5. Antibiotics sensitivity test

The antibiogram of the isolates to selected conventional antibiotics was determined by the disc diffusion method. Using antibiotic-impregnated paper discs (Medicare Nig. Ltd.) containing the following antibiotics: Pefloxacin, gentamicin, Ampliclox, erythromycin, Zinnacef, Amoxacillin, Rocephin, Ciprofloxacin, streptomycin and SeptrinSterile Petri dishes were seeded aseptically with 1 ml each of 18 h old pure cultures of the test organisms each while about 15 ml of sterilized Muller-Hinton agar was poured aseptically on the seeded plates. The culture was first standardized using spectrophotometer and plate count methods at 2.0 ×104 cfu/ml. McFarland standard at 540 nm (0.050 spectrophotic reading) was used. The plate were swirled carefully for even distribution and allowed to gel. With the aid of sterile forceps the antibiotics discs were placed firmly on solidified plates and incubated for 24 h at 37°C. After incubation, zones of inhibition were measured in millimeter (mm). The experiment was carried out in triplicate (CSLI, 2014).

3.0. RESULTS

3.1 Types of bacteria isolated from different wound samples

Different strains of bacteria were isolated from various wound samples. These bacterial species include; *S. aureus*, *P. aeruginosa*, *Proteus* spp., and *E. coli*. The morphological and biochemical characteristics can be found on Table 1.

3.2. Frequency of occurrence of bacteria isolated from CS wound samples

The most frequently isolated bacteria was *S. aureus* (13; 41.9%) followed by *P. aeruginosa* (8; 25.8%) and *Proteus* spp. (8; 25.8%) while *E. coli* (2; 6.4%) was the least isolated bacteria. This observation can be seen in Figure 1.

3.3. Antibiotics sensitivity pattern of Gram-positive bacteria isolated from CS wound samples

The only Gram-positive bacteria isolated from the CS wound swab sample was *S. aureus*. Streptomycin, rifampin, ciprofloxacin and levofloxacin are the most active antibiotics against *S. aureus*. Some strains of *S. aureus* isolated exhibited multiple resistance to the antibiotics used (amoxicillin, nalidixic acid, streptomycin, eerythromycin, chloramphenicol, ampiclox and gentamycin). These observations are displayed in Figures 2.

Table 1: Biochemical characteristics of isolated bacteria from different sources

S/N	Cell shape (Arrangement)	Gram Reaction	Catalase	Coagulase	Oxidase	Citrate	H_2S	Mannitol	Lactose	Sucrose	Dextrose	Glucose/e	Probable Organisms
1	Bacilli (clustered)	-	+	-	-	-	-	+	+	-	+	+	*E. coli*
2	Bacilli (clustered)	-	+	-	+	-	+	+	+	+	+	+	*P. aeruginosa*
3	Cocci (clustered/chain)	+	+	+	-	+	+	+	+	+	+	+	*S. aureus*
4	Cocci (clustered)	-	+	-	-	+		-	+	+	+	+	*Proteus* spp.

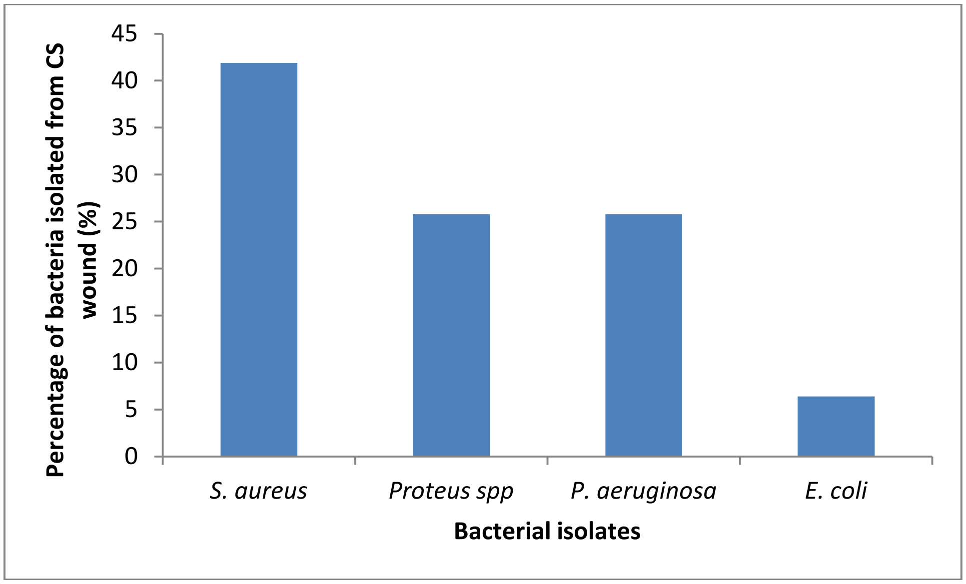

Figure1: Frequency of occurrence of bacteria isolated from CS wounds samples

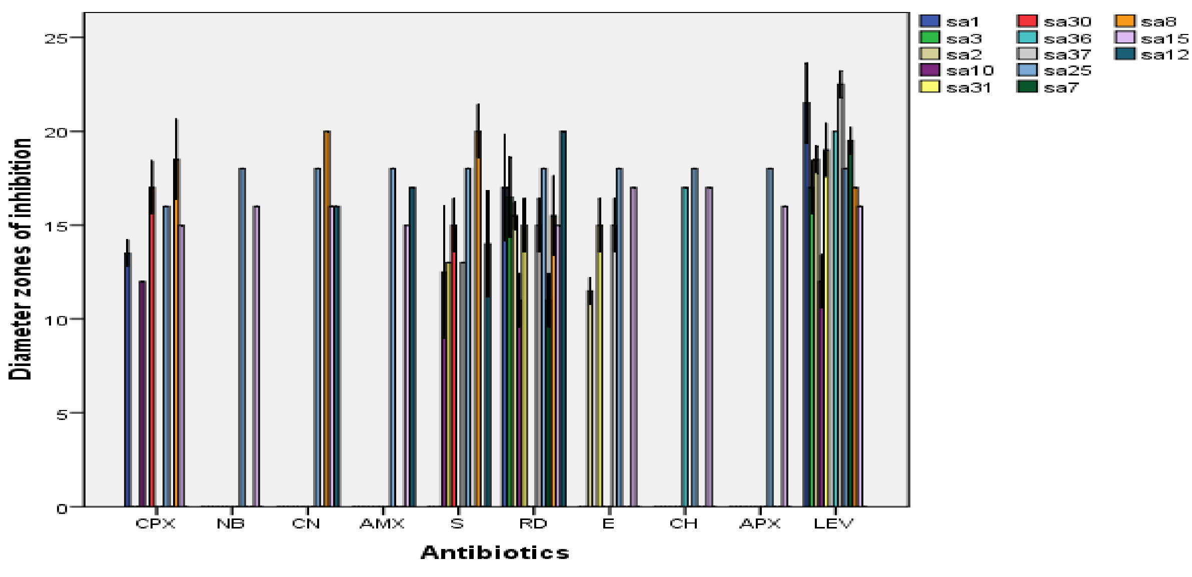

Figure 2: Antibiotics sensitivity pattern of Gram positive isolates

KEY: CH-Chloramphenicol; APX-Ampiclox; LEV-Levofloxacin; CPX- Ciprofloxacin, CN-Gentamicin, NB-Norfloxacin; AMX-Amoxil, S-Streptomycin; RD-Rifampicin; E-Erythromycin sa1 – sa3, sa7, sa8, sa12, sa15, sa12, sa25, sa30, sa31 sa36 and sa37 - *S aureus* isolated from CS wound samples

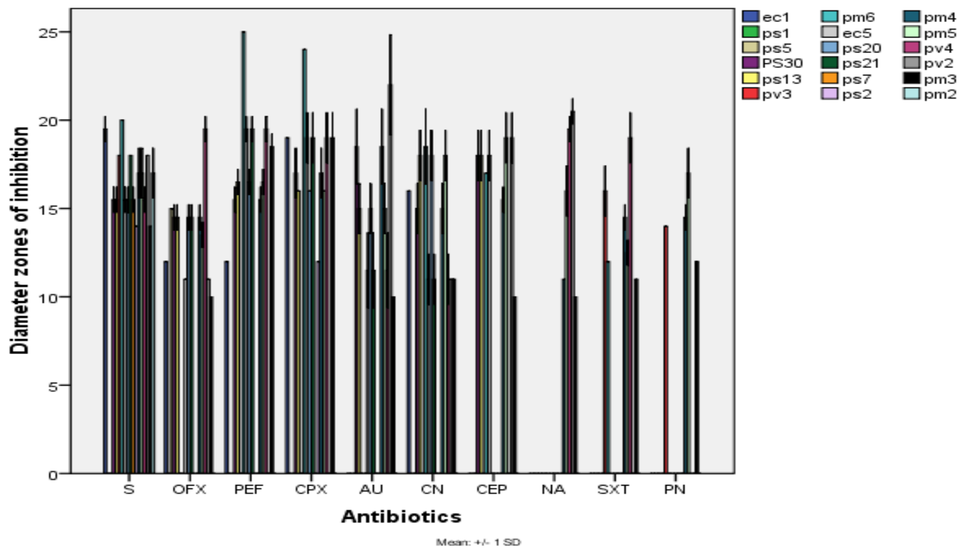

Fig3: Antibiotics sensitivity pattern of Gram negative bacteria isolatesd from CS wound samples.

KEY: OFX-ofloxacin, PEF-Pefloxacin, CPX-ciprofloxacin, AU-Augmentin, CN-gentamicin, S-Streptomycin, CEP- Cephem, NA-Nalidixic acid, SXT- Septrin, PN-ampicillin; ec1 and ec5- *E. coli* isolated from CS wound samples; ps5, ps30, ps1, ps13, ps7, ps2, ps20, ps21- *P. aeruginosa* isolated from CS wound samples; pv3, pm4, pm5, pv4, pv2, pm3, pm2- *Proteus* spp. isolated from CS wound samples

3.4. Bacterial isolated from CS wound swabs with multiple resistance to antibiotics

Tables 2 and 3 showed the isolates that displayed multiple resistant to the antibiotics used. *P. aeruginosa* (4; 50.0 %), and *Proteus* spp. (3; 37.5%) were resistant to aminoglycosides, penicillin, fluoroquinolones, β-Lactam/β-Lactamase inhibitor, aminoglycosides, cephem, quinolones, folate pathway inhibitor (Table 2). While, *S. aureus* (7; 53.8%) were resistant to aminoglycosides, penicillin, fluoroquinolones, ansamycins, macrolides and phenicols (Table 3). The isolated Gram-negative bacteria were highly resistant to septrin, ampicillin and cephem (Figure 4), while the isolated Gram-positive bacteria were highly resistant to norfloxacin, gentamycin, ampiclox, erythromycin and chloramphenicol (Figure 5).

Table 2: Percentage of Gram negative bacterial isolates that displayed multiple resistance to conventional antibiotics

S/N	Isolates	Number of resistant strains to different classes of antibiotics							REMARKS (%)
		Aminoglycosiddes (%)	Penicillin (%)	Fluoroquinolones (%)	β-Lactam/β-Latamase inhibitor (%)	Cephem (%)	Quinolones (%)	Folate pathway inhibitor (%)	
1	*P. aeruginosa* (n=8)	4(50)	4(50)	4(50)	4(50)	4(50)	4(50)	4(50)	MDR= 4(50)
2	*Proteus* spp (n=8)	3(37.5)	3(37.5)	3(37.5)	3(37.5)	3(37.5)	3(37.5)	3(37.5)	MDR= 3(37.5)

The zone of inhibition was interpreted as resistance, intermediate or susceptible according to the Clinical and Laboratory Standards Institute (CLSI), 2014 interpretative chart.

KEYS:

MDR- Multi Drug Resistant Bacteria

Table 3: Percentage of Gram positive bacterial isolates that displayed multiple resistance to conventional antibiotics

Isolates	Number of resistant strains to antibiotics						REMARKS (%)
	Aminoglycosides (%)	Penicillins (%)	Fluoroquinolones (%)	Ansamycins (%)	Macrolides (%)	Phenicols (%)	
1 *S. aureus* (n=13)	7(53.8)	7(53.8)	7(53.8)	7(53.8)	7(53.8)	7(53.8)	MDR= 7(53.8)

The zone of inhibition was interpreted as resistance, intermediate or susceptible according to the Clinical and Laboratory Standards Institute (CLSI), 2014 interpretative chart.

KEYS:

NMDR- Non Multi Drug Resistance;

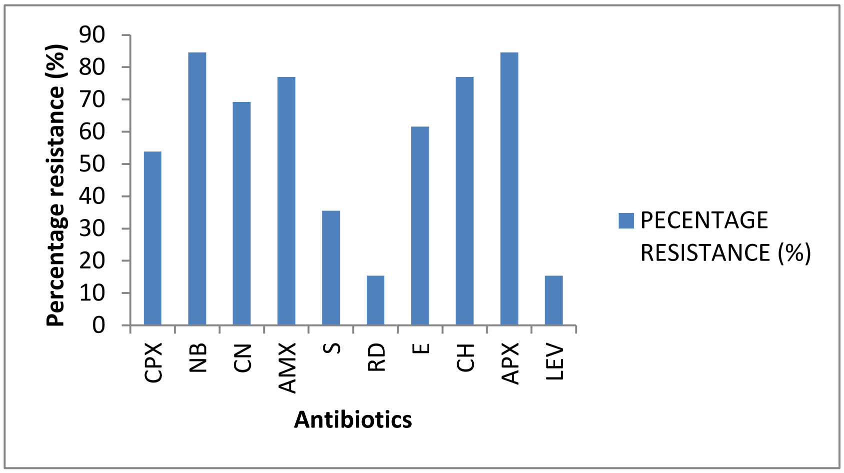

Figure 4: Percentage resistance of Gram-positive bacteria isolated from to antibiotics

The zone of inhibition was interpreted as resistance, intermediate or susceptible according to the Clinical and Laboratory Standards Institute (CLSI), 2014 interpretative chart.

KEY: OFX- Ofloxacin, PEF- Pefloxacin, CPX-ciprofloxacin, AU- Augmentin, CN- Gentamicin, S-Streptomycin, CEP- Cephem, NA- Nalidixic acid, SXT- Septrin, PN-Ampicillin

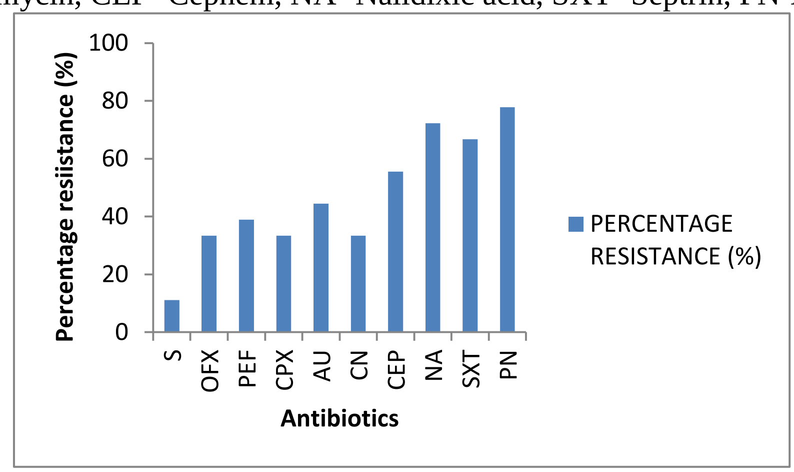

Figure 5: Percentage resistance of Gram-negative bacteria isolated from to antibiotics

The zone of inhibition was interpreted as resistance, intermediate or susceptible according to the Clinical and Laboratory Standards Institute (CLSI), 2014 interpretative chart.

KEY: OFX- Ofloxacin, PEF- Pefloxacin, CPX-ciprofloxacin, AU- Augmentin, CN- Gentamicin, S-Streptomycin, CEP- Cephem, NA- Nalidixic acid, SXT- Septrin, PN-Ampicillin

DISCUSSION

The type of bacteria isolated from the wound sample collected from Caesarean section of women attending Mother and Child hospital in Akure Ondo state were *S. aureus*, *Pseudomonas* sp., *Proteus* sp. and *Escherichia coli.* This is in accordance with the report of Agboeze *et al.* (2014). The observation that *S. aureus* (42%) was the predominant bacteria isolated is in agreement with the report of Valarmathi *et al.* (2013). The high incidence of *S. aureus* could be due to their abundance on human skin as normal flora. However, its abundance can pose a serious threat as it can cause series of infection when it gets to the mucosal part of the body especially during wound ailment. It has been implicated with infections such as impetigo, cellulitis, bacteremia and septicaemia among others (Vos, 2012; Kumar *et al.*, 2007). The presence of *E. coli* and *Proteus* species can be due to contamination of wound with patient's endogenous flora (Opalekunde *et al.*, 2014). The presence of *E. coli* on the wound is a major threat as it can result to bactermia if it gets to the blood vessels and also prolong the stay in the hospital and increase hospital bills (Valarmathi *et al.*, 2013).

The presence of *Pseudomonas* sp is a great threat to mothers attending Mother and Child Hospital as it has emerged as one of the most important pathogen during the past two decades. It causes between 10% and 20% of nosocomial infections. The most serious infections include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia (Gerald *et al.,* 2016). The resistance of some strains of *Proteus* spp. to septrin, ampicillin and nalidixic acid is in agreement with Mwambete and Rugemalila (2015) who stated that *Pr. mirabilis* had 50% resistant to antibiotics treatment. Forty percent of the isolated *S. aureus* displayed multiple resistance to conventional antibiotics used (streptomycin (Aminoglycosides), Norfloxacin (Fluoroquinolones), Amoxicillin (Penicillin), Ampiclox (Penicillin), Levofloxacin (Fluoroquinolones), chloramphenicol (Phenicols), Gentamycin (Aminoglycosides, erythromycin (macrolides) and ciprofloxacin (Fluoroquinolones). This result corroborates the report of Opalekunde *et al.* (2014) and Mwambete and Rugemalila (2015). The resistance observed in *Staphylococcus aureus* could be attributed to irrational use of antibiotics for conditions that may not clinically indicate their use, over the counter sales of antibiotics in pharmacies without prescription by authorized practitioners, some drug formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them (Opalekunde *et al.*, 2014).

This resistance displayed is a great threat to the health of these post natal patients resulting to prolong stay in the hospital. The life of the new baby is likewise at risk due to the exposure of the immunodefficient babies to infectious bacteria. Most of the isolated strains are susceptible to Rifampicin, streptomycin, ampicillin, levofloxacin and ofloxacin in contrast to Agboeze *et al.* (2014)

CONCLUSION AND RECOMMENDATION

Bacteria isolated from Caesarean section wound swabs are pathogenic bacteria. The resistance of the isolated bacteria to most of the antibiotics tested is of major concern because so many complications that can result after CS delivery. There is therefore the need to source for alternative therapy for the treatment of wounds after CS delivery to prevent infection and wound sepsis that may jeopardize the health of the woman that has just given birth. Hygienic condition should be more intensified among the hospital workers so as to minimize nosocomial infections and any other infectious diseases

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