Susceptibility Pattern of Bacteria Isolated from Postoperative Wounds of Hysterectomy Patients in Catholic Hospitals at Umuahia, Abia State, Nigeria

[1]**Nwankwo I.U,** [2]**Edward K.C,** [3]**Nwoba, C.N and** [4]**Nwaka, S.O.**
[1,2,4] Department of Microbiology, Micheal Okpara University of Agriculture, Umudike
[3] Medical Laboratory Department, Micheal Okpara University Of Agriculture, Umudike, Medical Centre.
Correspondence Author: immaugo@yahoo.com

Abstract: The study identified the bacterial isolates associated with the postoperative wound of women that underwent a hysterectomy and determined their susceptibility pattern to some antibiotics. Sixty (60) specimens of a postoperative wound from patients that underwent hysterectomy were aseptically collected and cultured using standard microbiological procedures. The isolates were identified using Gram stain and biochemical methods, and disc diffusion method was used to test for the susceptibility pattern of the isolates. Six bacterial isolates, namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* specie, *Klebsiella* species, and *Proteus* species, were isolated. Amongst the six isolates, *Pseudomonas aeruginosa* had the highest frequency of occurrence 12(29.3%), followed by *Staphylococcus aureus* 10(24.4%), *Klebsiella* specie 8(19.5%), *Escherichia coli*, 5(12.5%) while the least was recorded against *Proteus* specie 2(4.9%). Among the Gram-positive bacteria, the high level of resistance was recorded by *Streptococcus* specie (80%) against penicillin, followed by *Staphylococcus aureus* (70%). In comparison, the least level of resistance was observed with ampicillin (25%) against *Streptococcus* specie. The zone of inhibition of the antibiotics against the isolates ranges from 8.7–17.3mm for the Gram-negative and 8.2-17.6mm for Gram-positive isolates. Streptomycin and Gentamicin had the highest inhibition (17.3mm) against Gram-negative, while penicillin produces the highest zone of inhibition against the Gram-positive isolates. The study identifies the pronounce resistance of isolates to commonly used antibiotics, which suggested the need for rational use of the drugs to prevent the emergence of multi-drug resistant strains. Appropriate infection control measures and sound antibiotic policy are necessary to reduce postoperative wound infections.

Keywords: Susceptibility pattern, Postoperative wounds, Hysterectomy, Bacteria isolates.

INTRODUCTION

Surgical and open wounds are commonly encountered in clinical practice (Biadgleyne *et al.*, 1990). Surgical site infections are the most common nosocomial infections (Mahy and Meulen (2007); Biadgleyne *et al.* (2009). They are a significant source of postoperative morbidity resulting in more extended hospitalization, increased cost, and increased postoperative mortality (Agboeze *et al.*, 2014).

Postoperative wound infections are those wounds acquired during surgical procedures (Barie *et al.,* 2012). Postoperative wound infections can be caused by two significant sources: exogenous and endogenous bacteria (Bowler *et al.,* 2001). The probability of wound infections largely depends on the patients' systemic host defenses, local wound conditions, and microbial burden (Lilian *et al.*, 2005).

All surgical wounds are contaminated by both pathogens and body commensals (Anguzu and Olita, 2007). Still, the development of infection in the site depends on the complex interplay of many factors (Olisen, 2008). These may be microbial virulence (Bowler *et al.*, 2007), patients risk factor like diabetes, cigarette smoking, obesity and coincident remote site infections or colonization (Reichman and Green-berg, 2009), and operation-related risk factors including prolonged hospital stay before surgery, duration of the process, tissue trauma, inadequate hemostasis and foreign material in the wound (Rubin, 2006). Meanwhile, the organism that would invade the tissue depends on the injury (Thomas *et al.*, 1998). The microbial pathogens are, therefore, brought to the wound site by direct contact, self-contamination, and airborne (Prakash, 2010).

Hysterectomy is one of the most common gynecologic operations (Miranda *et al.,* 1993; Merrill, 2013).

Fibroids of the uterus and gynecologic malignancies are frequent indications for this type of surgery. Infection at the surgical site after abdominal hysterectomy is still a common complication, with an incidence of 6-12% (Armour *et al*., 2007).

The major risk factors for developing surgical site infections after a hysterectomy include older age, poor general health condition, prolonged hospitalization, extended surgery, and extensive bleeding (Bach *et al*., 2002).

Several studies have shown *Staphylococcus aureus*, *Mycoplasma*, *Escherichia coli*, and anaerobicbacteria as being the causative pathogens (Kobamatsu *et al.,*1991; Miranda *et al.,* 1993; Bikowski 1999; Bowler *et al.,* 2001; Shrestha and Basnet,2019). Antibiotic prophylaxis, usually involving an agent belonging to first or second-generation cephalosporins (preferably with an additional anaerobic coverage),has been shown to reduce the risk of surgical site infection after hysterectomy (Houang, 1991;Bikowski, 1999; Kamat *et al.,*2000; Lofgren *et al.,*2004).

However, Mangram (1999) reported that about 70% of the bacteria that causes infections in the hospital are resistant to at least one of the drug most commonly used for the treatment of diseases caused by them (Raza and Ranabhat, 2013). Some organisms are immune to all approved antibiotics; hence, they can only be treated with experimental and potentially toxic drugs. The current spread of multi-drug resistance bacteria pathogens has added a new dimension to the problem of wound infections (Lee *et al*., 1990). This is significantly worse in resource-poor countries where the sale of antibiotics is under poor control (Kleven, 2007).

For qualitative health care delivery to patients with infected wounds, a regular bacteriological review of infected wounds is necessary. The study aimed at identifying the bacterial species associated with post–operated wounds of hysterectomy patients and evaluating the susceptibility pattern of the bacterial isolated to some commercial antibiotics.

MATERIALS AND METHODS

After obtaining ethical clearance from the hospital ethical committee and filling of the consent form by all the patients involved in the study, 60specimens of a postoperative wound from hysterectomy patients from patients that attended Madona Catholic Hospital, Umuahia, and Mercy Specialist Hospital Ahiaeke were aseptically collected with the aid of sterile disposable moistened swab sticks and transported to the laboratory of the Department of Microbiology Michael Okpara University of Agriculture, Umudike for microbiological analysis.

The isolation and identification of bacteria from the postoperative wound infections were carried out following microbiological procedures that involved streaking on MacConkey agar and incubated at 37^{o}C for 24 hours and identified using Gram staining and biochemical reactions as described by Cheesebrough (2006). The antibiotics sensitivity test was assayed on Mueller Hinton agar by Kirby-Bauer's disk diffusion method, as described in the clinical and laboratory standard institute (CLSI) (2014).

RESULTS

Six bacteria isolates (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Klebsiella pneumoniae,* and *Proteus vulgaris*) were isolated from samples of patients who underwent a hysterectomy. All the samples (100%) showed growth of aerobic bacteria. All the positive samples showed mixed infection, and a total of 41 bacteria were isolated. *Pseudomonas aeruginosa* 12(29.3%). *Staphylococcus aureus* 10 (24.4%), *Klebsiella pneumoniae* 8 (19.5%), *Escherichia coli* 5(12.2%) *Streptococcus pyogenes* 4 (9.8%) and *Proteus vulgaris* 2 (4.9%) (Table 1).

The antimicrobial susceptibility patterns of Gram-positive and Gram-negative bacteria isolates are presented in Table 2.

The predominant Gram-positive isolate, *Staphylococcus aureus,* showed a high level of resistance to Gentamicin 7 (70.0%), Ampicillin 6 (60.0%), and Tetracycline 5 (50.0%). It was more sensitive to Penicillin and Ciprofloxacin as only 3 (30.0%) out of the ten isolates were resistant to these drugs. All the *Streptococcus* species isolated were resistant to Gentamicin 4(100%). Amongst the Gram-negative, *E. coli* and *Proteus* species exhibited 100% sensitivity to Ampicillin and Gentamicin, respectively. Up to 80% of *E.coli* isolated were resistant to streptomycin. *Pseudomonas* species had 100% resistance to penicillin, and 62.5% of *Klebsiella* species isolated were resistant to Ciprofloxacin.

The diameter of the zone inhibition of the antibiotics against the isolates from hysterectomy wound infection was presented in Table 3. The diameter of the zone of inhibition of the antibiotics against the Gram-negative bacteria isolates ranges from 8.7 – 17.3mm. Streptomycin and Gentamicin recorded the highest zone of inhibition (17.3mm, respectively) against *Klebsiella* species. The least zone of inhibition (8.7mm) was observed with Tetracycline and Ampicillin against *Pseudomonas aeruginosa*. For the Gram-positive isolates, the diameter of the zone inhibition of the antibiotics ranges from 8.2 – 17.6mm. The highest zone of inhibition (17.6mm) was observed with Penicillin against *S.aureus*. It was followed by streptomycin (16.2mm) against the same organism, while the least was recorded against ampicillin (8.2mm) on *S. aureus*.

Table 1: Isolated bacteria from postoperative wounds of Hysterectomy patients and their frequency of occurrence

Bacteria isolates	No. of isolates	% occurrence
Escherichia coli	5	12.5
Staphylococcus aureus	10	24.4
Pseudomonas aeruginosa	12	29.3
Streptococcus pyogenes	4	9.8
Klebsiella pneumoniae	8	19.5
Proteus vulgaris	2	4.9
Number of samples studied=60		
Total	**41**	**100**

Table 2: Percentage Resistance Pattern of Bacterial Isolates from Patients with Postperative Hysterectomy wound

Bacteria isolates	N	No of Resistant pathogens to Antibiotics (%)					
		Pen	Cip	Strep	Gent	Tet	Amp
Staphylococcus aureus	10	3(30.0)	3(30.0)	4(40.0)	7(70.0)	5(50.0)	6(60.0)
Streptococcus pyogenes	4	2(50.0)	3(75.0)	2(50.0)	4(100)	1(25.0)	2(50.0)
Escherichia coli	5	3(60.0)	2(40.0)	4(80.0)	2(40.0)	3(60.0)	0(0)
Pseudomonas aeruginosa	12	12(100)	4(35.0)	3(25.0)	8(66.7)	2(16.7)	7(58.0)
Klebsiella pneumoniae	8	2(25.0)	5(62.5)	2(25)	2(25.0)	4(50.0)	4(50.0)
Proteus vulgaris	2	1(50.0)	1(50.0)	1(50.0)	0(0)	1(50.0)	1(50.0)

***Key*:** n= number of isolates; Pen = penicillin, Cip= ciprofloxacin, Strep= streptomycin, Tet= Tetracycline, AMP= Ampicillin, Gent: Gentamicin

Table 3: Antibiotic susceptibility pattern of the Gram-negative bacterial isolates from patients with hysterectomy wound infection

Isolate code	Antibiotics/Zone of Inhibition (mm)					
	Pen	**Cip**	**Strep.**	**Gent.**	**Tet.**	**Amp.**
Ec_1	13.5	16.2	0.0	10.4	0.0	12.3
Ec_2	0.0	9.4	0.0	11.2	0.0	14.2
Ec_3	0.0	0.0	14.3	0.0	11.4	17.4
Ec_4	11.3	17.2	0.0	14.1	10.2	13.1
PA_1	0.0	9.5	0.0	10.2	0.0	12.2
PA_2	0.0	12.3	10.0	9.3	0.0	10.5
PA_3	0.0	0.0	8.8	0.0	9.8	0.0
PA_4	0.0	9.7	11.0	0.0	9.3	0.0
PA_5	0.0	0.0	12.2	9.4	9.5	0.0
PA_6	0.0	0.0	9.5	0.0	10.0	8.7
PA_7	0.0	10.4	0.0	10.6	8.9	0.0
PA_8	0.0	9.4	10.3	0.0	9.0	0.0
PA_9	0.0	0.0	10.5	0.0	8.7	0.0
PA_{10}	0.0	11.2	13.2	0.0	9.3	9.2
PA_{11}	0.0	12.0	10.1	0.0	9.8	0.0
PA_{12}	0.0	13.3	0.0	0.0	10.0	9.2
Ks_1	10.2	12.2	9.8	17.3	15.4	0.0
Ks_2	12.4	10.4	13.0	14.4	14.7	9.6
Ks_3	0.0	0.0	0.0	0.0	0.0	0.0
Ks_4	9.8	10.7	8.9	12.6	11.0	0.0
Ks_5	11.3	0.0	12.6	11.9	11.5	10.2
Ks_6	11.1	0.0	17.3	13.7	10.3	9.7
Ks_7	13.2	0.0	15.4	16.6	12.7	8.9
Ks_8	0.0	0.0	0.0	0.0	0.0	0.0
Ps 1	0.0	0.0	9.8	10.7	0.0	13.0
Ps 2	9.7	11.7	0.0	12.2	11.6	10.2

Key: Pen = penicillin, Cip= ciprofloxacin, Strep= streptomycin, Tet= Tetracycline, AMP= Ampicillin, Gent: Gentamicin,Ec = *E.coli*: PA = *Pseudomonasaeruginosa*; Ks = *Klebsiella* species; Ps = *Proteus*, species. Arabic numerical = serial number of the isolates.

Table 4: Antibiotic susceptibility pattern of the Gram-positive isolates from patientswith hysterectomy wound infection

Isolate code	Antibiotics/Zone of Inhibition (mm)					
	Pen	**Cip**	**Strep.**	**Gent.**	**Tet.**	**Amp.**
SA_1	14.5	13.1	0.0	0.0	9.7	0.0
SA_2	17.6	0.0	16.2	0.0	11.6	8.2
SA_3	0.0	11.4	10.4	0.0	0.0	10.7
SA_4	10.2	9.7	0.0	13.3	0.0	0.0
SA_5	0.0	10.5	11.3	0.0	0.0	10.3
SA_6	9.8	0.0	12.1	0.0	10.2	9.4
SA_7	11.6	14.2	0.0	0.0	0.0	0.0
SA_8	0.0	0.0	11.6	10.1	12.3	11.6
SA_9	12.3	12.6	0.0	0.0	11.4	0.0
SA_{10}	10.7	0.0	12.4	14.2	0.0	12.3
SP_1	0.0	10.8	0.0	9.6	8.7	0.0
SP_2	0.0	0.0	10.6	12.1	11.6	9.3
SP_3	14.2	0.0	12.4	16.4	0.0	11.7
SP_4	11.7	0.0	0.0	12.8	10.3	0.0

Key: Pen = penicillin, Cip= ciprofloxacin, Strep= streptomycin, Tet= Tetracycline, AMP= Ampicillin, Gent: Gentamicin, SA = *Staphylococcus aureus*, SP = *Streptococcus pyogenes*, Arabic numerical = serial number of the isolates

DISCUSSION

Hysterectomy is a standard gynecologic procedure, and wound infections are not rare occurrences. The use of prophylactic antibiotics is successful in decreasing the incidence of wound infections in women undergoing abdominal hysterectomy.

As the antimicrobial resistance patterns of the bacteria isolates keep changing, and evolving with time and place (Sani *et al*., 2012). This study was conducted to assess the current status of bacteria pathogens involved in postoperative wound infection from hysterectomy cases and their sensitivity pattern.

This study observed that all samples (100%) were culture positive showing the growth of aerobic bacteria on culturewas comparable with the report of Rubin (2006), where 76.6% of samples were culture positive.

Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and *Proteus vulgaris* were the bacteria species isolated from this study. These findings resemble the report of Agboeze *et al.* (2014) and Preethishree *et al.* (2017), where *S. aureus*, *P. aeruginosa*, *Proteus* species, and *E. coli* were isolated from postoperative wound infection. This difference in the type of bacteria species isolated from postoperative infection may be due to variation in common nosocomial pathogen inhabitant, the difference in policy of infection control and prevention between countries and hospitals, and the study design used in the researches (Yerushalmy *et al*., 2008; Ahmed and Wasti, 2001).

The observation that *Pseudomonas aeruginosa* (29.3%) was the predominant bacteria isolated is in disagreement with the report of Nitin *et al.* (2013) and Preethishree *et al*. (2017), where the highest occurrence was recorded with *Staphylococcus aureus*. The presence of *Pseudomonas specie i*s an excellent threat to patients as it has emerged as one of the most important pathogen during the past two decades. It causes between 10% and 20% nosocomial infections. The most severe infections include malignant external otitis, endophthalmites, endocarditis, meningitis, pneumonia, and septicemia (Gerald *et al*., 2016). The second predominant bacterium was *Staphylococcus aureus*. Its predominance is evidentas it forms the bulk of normal flora of the skin and nails and could be inoculated into the incision during operation (Barie, 2012). Another reason for the high incidence of *S. aureus* in surgical wound infections, as observed in this study, could be attributed to the high rate of nasal carriage of this organism in patients and healthcare workers (Zorganie *et al*., 2002). This reason also explains the isolation of other bacteria of respiratory origin. The presence of *E. coli*and *Proteus* sp may be due to spread via fecal contamination of people, instruments, or other surfaces in the hospital environment (Bahar *et al.,* 2012).

Furthermore, it was observed from this study that Gram-negative organisms were predominantly the causative agents of surgical wound infection. This agrees with the report by Nitin *et al. (*2013).

More so, the antibacterial sensitivity testing of the isolates reveals that *S. aureus* showed increased resistance to commonly used antibiotics like Gentamicin (70%) and Ampicillin (60%), and it is in agreement with the report of Giacometti *et al*. (2000). The high level of resistance against these two antibiotics may be due to the increased use of these antibiotics in the selected ward for prophylaxis a postoperatively to prevent infections and empirical treatment for infected cases (Giacometti *et al.,* 2000). On the other hand, *Streptococcus* strains were susceptible to Gentamicin (100%), similar to what was recorded by (Agboeze *et al.,* 2014).

Pseudomonas aeruginosa also showed a total resistance to penicillin in this study and is contrary to what was reported by Gautan *et al*. (2012) and Amoran *et al*. (2013), where *Pseudomonas aeruginosa* showed maximum sensitivity by penicillin.

Pseudomonas aeruginosa, on its own, has been of significant threat in surgical site infection due to its records of staggering resistance to extended-spectrum antibiotics (Bahar *et al.,* 2012). The organism has defied therapy, even with many drugs in the last line of defense.

CONCLUSION:

Pseudomonas, aeruginosa, and *Staphylococcus aureus* were the most common bacteria isolated in a post-hysterectomy wound infection in the study. Streptomycin was the most effective drug against the Gram-positive bacteria. In contrast, streptomycin and tetracycline were the most effective antibiotics against some Gram-negative bacteria in the study. For individual surgical sites, the local infecting organism and their sensitivity pattern should be taken into account when formulating prophylaxis, as well as empirical therapy guideline for such individual patients.

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