Original Research Article

ANTIBIOGRAM AND PLASMID MEDIATED RESISTANCE IN BACTERIA ISOLATED FROM INFECTED WOUNDS

ABSTRACT

Bacterial infection of wound plays an important role in the development of chronicity and delayed healing. In this study, a total of 50 wound swabs were aseptically collected from patients attending specialist hospital Jimeta Yola, Adamawa State and were screened for bacteria. The isolates were identified using Gram-staining and biochemical tests. Eight different bacterial species were identified with *Staphylococcus aureus* having the highest occurrence with 11(26.19%), followed by *Escherichia coli* 8(19.05%), *Klebsiella pneumoniae* 6(14.29%), *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* 5(11.9%), *Proteus vulgaris* and *Streptococcus pyogenes* 3(7.14%) and lastly, *Bacillus subtilis* with 1(2.38%). Antibiotic susceptibility test using Kirby-Bauer disk diffusion method revealed that most of the Gram-positive isolates significantly resisted oxacillin, penicillin and amoxicillin. Most Gram negatives significantly resisted septrin, chloramphenicol, amoxicillin, augmentin and pefloxacin. Ciprofloxacin was 100% effective against both Gram positive and Gram-negative isolates. Plasmid curing of MDR isolates using 10% sodium dodecyl sulphate (SDS) revealed that resistance to penicillin, oxacillin, amoxicillin, augmentin and pefloxacin were plasmid borne whereas chloramphenicol and septrin (trimethoprim) were not.

Key words: Wound, Bacteria, Antimicrobial resistance, Plasmid curing

1.0 INTRODUCTION

Bacterial introduction into particularly, surface wounds is somehow inevitable consequent to their direct exposure to the external environment. Infection and colonization of wound is a major challenge to wound care specialists accounting for high morbidity and mortality in recent years. A plethora of microorganisms have been found to associate with wounds most of which originate from either the environment, the patient's flora, medical and surgical devices, or from other humans [1]. However, the development of wound sepsis is multi factorial, as the integrity of the type of microorganisms involved, their synergy, their pathogenicity, their virulence, nature of the wound, use of antibiotics and the immune competency of the host are important determining factors [2]. A number of studies conducted on wound infection reported that bacterial colonization of wound sites by pathogens contributes substantially to its chronicity which could consequently be burdensome not just to the patients themselves but also the health personnel due to the overwhelming effort required in the treatment and care of the wounds [3, 4].

Several studies have been conducted from different parts of the globe on wound microbiome with their antibiogram and *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Proteus mirabilis* have been identified to be the most prevalent bacterial in

wounds [5, 6, 7, 8]. However, the distribution of bacteria in wounds and their antimicrobial susceptibility have shown a substantial geographic variation [9, 4]. Reports have shown that chronic wound infections are accompanied by a series of devastating events particularly when the number of microbes begins to increase and spread throughout the body through the blood stream and hence overwhelms the host's immune system causing systemic symptoms such as fever, chills and tachycardia [10]. Bowler *et al* [11] reported that wound fails to heal in the event of infection and the patient suffers increased trauma, treatment costs rise, and general wound management practices become more resource demanding.

Bacterial resistance to orthodox antibiotics is now a global challenge with increase reports each year as non-pathogenic strains acquire resistance through horizontal gene transfer. An infected wound is a home for a diverse number of microorganisms and as such, a complex microbial community with high interactions including exchange of genetic material is established.

Studies from different parts of the globe indicated that bacterial isolates associated with wound infections exhibits high level of resistance to multiple antibiotics [9, 12, 4]. Therefore, there is need for the knowledge of different bacteria associated with wound infection and their antibiotic susceptibility pattern to aid in the appropriate choice of treatment that would enhance the healing process of wound.

2.0 MATERIALS AND METHOD

Sample Collection

A total of 50 patients with wound infections during the study period were enrolled through convenient sampling techniques as described by [13]. Wound secretions/pus were collected from each study participant using sterile cotton swab sticks. Each specimen was immersed in sterile peptone water in a labeled bijou bottle and transported to the laboratory for microbiological analysis.

Isolation and Identification of Isolates

Each of the samples collected was inoculated on MacConkey (MCA) agar and blood agar (BA) plates using streak plate method. All the plates were incubated aerobically at 37°C for 24 hours. Plates without growth were further incubated for 24 hours. Then cultural characteristics including colonial morphology, coloration, and hemolysis were observed and recorded. Morphologically distinct colonies were further sub-cultured on freshly prepared labeled Nutrient agar plates to obtain pure cultures of the isolates and incubated for 37°C for 24 hours. All the isolates were identified through Gram-Staining and biochemical tests viz; methyl red, Vorges-Proskauer, indole, citrate, catalase, oxidase, coagulase, urease and H_2S/motility test as described in standard operating procedure (SOP) Bacteriology, Indian Council for Medical Research (ICMR) [14].

Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was carried out on each isolate using Kirby-Bauer disc diffusion method on Muller-Hinton agar (MHA) using standard method as recommended by Clinical Laboratory Standard Institute (CLSI) [15].

Plasmid Curing

Isolates exhibiting resistance to multiple drugs were subjected to plasmid curing using 10% sodium dodecyl sulfate (SDS) as described by Zaman *et al* [16].
Ten percent (10%) SDS was prepared by diluting 5g of SDS powder in 45ml of sterile nutrient broth, such that 1/10 of the required volume is needed to give the final concentration.
Overnight culture of each isolate was incubated in nutrient broth at 37˚C for 24 hours. Each isolate was diluted to 10^4cells/ml from which 0.5ml was added to 4.5ml nutrient broth containing the SDS making the final cell density and SDS concentration to be 10^3cells/ml and 10% respectively. The tubes were incubated for 48 hours at 37˚C. The turbidity of each cured broth culture was again adjusted to 0.5 McFarland standard and 0.1ml of each culture was spread unto Mueller- Hinton agar plate and a nutrient agar plate (which served as control). Antibiotic susceptibility test was carried out on the Mueller-Hinton agar plates. For each of the cured isolates, the two plates were incubated at 37˚C for 24hours and observed for cured cells.
All isolates that exhibit growth on normal nutrient agar but showed considerable zone of growth inhibition around the antibiotic discs on the Mueller-Hinton agar plates were considered as possible cured isolates.

RESULTS

Out of the 50 wound swabs collected, 36 samples were having bacterial growth after overnight incubation. Overall, 42 different bacterial isolates were obtained out of which 8 different species were identified viz; *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis and Staphylococcus epidermidis*. *Staphylococcus aureus* has the highest frequency with 11(26.19%), followed by *E. coli* (19.05%), *K. pneumoniae* 6(14.29), *S. epidermidis* and *P. aeruginosa* with 5(11.9%) each, *P. vulgaris* and *S. pyogenes with* 3(7.14%) each, and lastly, *B. subtilis* has the least occurrence with only 1(2.38%) (Table 1).

Antimicrobial resistance pattern of Gram-positive isolates revealed that *S. aureus 1 and 2,* and *S. pyogenes 1* exhibited highest resistance to antibiotics resisting 6(50%) out of 12 antibiotics. The most susceptible isolates were *S. epidermidis 1, 2, 3 and 5* resisting only 1(8.33%) antibiotic each (Table 2).

The most resistant Gram-negative isolates to multiple antibiotics were *P. vulgaris 1,* and *P. aeruginosa 3 & 6,* with 6(60%) resistance out of 10 antibiotics, followed by *E. coli 7&8* and *K. pneumoniae 4* with 5(50%) resistance. *E. coli 4&6* were the most susceptible isolates with 2(20%) resistance (Table 3).

Oxacillin, penicillin and amoxicillin were the most resisted antibiotics among Gram Positives with 18(90%), 14(70%) and (14%) resistance respectively. Whereas all the isolates were more susceptible to ciprofloxacin with 100% susceptibility, followed by Cloxacillin and gentamycin both of which have 18(90%) (See Table 4).

The most resisted antibiotics among Gram negative isolates were Chloramphenicol with 16(72.73%), followed by Augmentin and pefloxacin with 13(59.09%) both, Septrin 12(54.55%)

and amoxicillin 10(45.45%). Whereas they are more susceptible to Ciprofloxacin with 22(100%), followed by Sparfloxacin 16(72.73%), gentamycin 13(59.09%) and Tarivid and Streptomycin both of which have 11(50%) susceptibility (See Table 6).

Plasmid curing among Gram positive bacteria indicated that resistance to oxacillin, penicillin and amoxicillin were plasmid borne as the isolates later became susceptible to the antibiotics after curing (Table 7).

Plasmid curing among Gram negatives indicated that resistance to augmentin, amoxicillin and pefloxacin were plasmid borne. Whereas resistance to Septrin and chloramphenicol were not plasmid borne (See Table 8).

Table 1: Distribution of Bacterial Species among Wound Samples

S/N	Organisms	Frequency	Percentage (%)
1	*Bacillus substilis*	1	2.38
2	*Escherichia coli*	8	19.05
3	*Klebsiella pneumonia*	6	14.29
4	*Proteus vulgaris*	3	7.14
5	*Pseudomonas aeruginosa*	5	11.90
6	*Staphylococcus aureus*	11	26.19
7	*Staphylococcus epidermidis*	5	11.90
8	*Streptococcus pyogenes*	3	7.14
	Total	42	100

Table 2: Antimicrobial Resistance Pattern of Gram-positive Isolates

S/N	Isolates	Antibiotics											Percentage Resistance
		VAN	OXA	CXC	PEN	E	TE	C	CRO	AML	CN	CIP	SXT	
1	*B. substilis*	S	S	S	S	R	S	S	S	R	S	S	R	3 (2 5 %)
2	*S. aureus 1*	I	R	S	R	R	R	S	S	R	I	S	R	6 (5 0 %)
3	*S. aureus 2*	S	R	S	R	R	R	I	S	R	S	S	R	6 (5 0 %)
4	*S. aureus 3*	S	R	I	R	S	R	S	S	R	S	S	S	4(33.33%)
5	*S. aureus 4*	S	R	S	R	S	R	S	I	R	S	S	S	4(33.33%)
6	*S. aureus 5*	S	R	S	R	S	R	S	S	R	S	S	S	4(33.33%)
7	*S. aureus 6*	I	R	S	R	S	S	S	S	R	S	S	S	3 (2 5 %)
8	*S. aureus 7*	S	R	S	R	S	S	S	S	R	S	S	I	3 (2 5 %)
9	*S. aureus 8*	S	R	S	R	S	S	I	S	R	S	S	S	3 (2 5 %)
10	*S. aureus 9*	S	R	S	R	S	S	S	S	S	S	S	S	2(16.67%)
11	*S. aureus 10*	I	R	S	R	S	S	S	S	R	S	S	S	3 (2 5 %)
12	*S. aureus 11*	S	R	S	R	S	S	S	I	S	S	S	S	2(16.67%)
13	*S. epidermidis 1*	S	R	I	S	S	S	S	S	S	S	S	S	1(8.33%)
14	*S. epidermidis 2*	S	S	S	S	S	S	S	S	R	S	S	I	1(8.33%)
15	*S. epidermidis 3*	S	R	S	I	S	S	S	S	S	S	S	S	1(8.33%)
16	*S. epidermidis 4*	S	R	S	S	S	S	S	S	R	S	S	S	2(16.67%)
17	*S. epidermidis 5*	I	R	S	S	S	S	I	S	S	S	S	S	1(8.33%)
18	*S. pyogenes 1*	S	R	S	R	S	R	S	R	R	S	S	R	6 (5 0 %)
19	*S. pyogenes 2*	S	R	S	R	S	R	S	S	S	R	S	S	4(33.33%)
20	*S. pyogenes 3*	S	R	S	R	S	R	S	S	R	S	S	S	4(33.33%)

Key: R= Resistance, I= Intermediate, S= Sensitive

Table 3: Antimicrobial Resistance Pattern of Gram-negative isolates

S/N	Isolates	Antibiotics										Percentage Resistance
		SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	
1	*E. coli 1*	S	R	S	S	R	R	S	R	S	I	4 (4 0 %)
	E. coli 2	S	R	S	S	R	R	S	R	S	S	4 (4 0 %)
	E. coli 3	S	R	S	S	R	R	S	S	I	I	3 (3 0 %)
	E. coli 4	I	S	S	S	S	R	S	R	S	S	2 (2 0 %)
	E. coli 5	R	S	R	S	S	R	S	S	S	S	3 (3 0 %)
	E. coli 6	S	R	S	S	I	R	S	S	S	S	2 (2 0 %)
	E. coli 7	S	S	R	S	S	R	S	R	R	R	5 (5 0 %)
	E. coli 8	R	S	R	S	S	R	S	R	R	S	5 (5 0 %)
2	*K. pneumoniae 1*	S	S	S	S	S	S	R	R	R	R	4 (4 0 %)
	K. pneumoniae 2	R	R	S	S	S	S	S	R	S	R	4 (4 0 %)
	K. pneumoniae 3	S	R	S	S	R	S	S	S	S	R	3 (3 0 %)
	K. pneumoniae 4	R	R	S	S	S	S	R	R	R	S	5 (5 0 %)
	K. pneumoniae 5	R	S	S	S	R	I	R	S	R	S	4 (4 0 %)
	K. pneumoniae 6	S	R	S	S	S	S	R	R	R	R	5 (5 0 %)
3	*P. vulgaris 1*	R	R	S	S	S	S	R	R	R	R	6 (6 0 %)
	P. vulgaris 2	S	R	R	S	S	S	S	R	S	S	3 (3 0 %)
	P. vulgaris 3	R	R	S	S	S	I	R	R	S	S	4 (4 0 %)
4	*P. aeruginosa 1*	R	R	S	S	R	R	S	S	S	S	4 (4 0 %)
	P. aeruginosa 2	R	R	S	S	R	R	S	S	S	S	4 (4 0 %)
	P. aeruginosa 3	R	R	S	S	R	R	S	S	R	R	6 (6 0 %)
	P. aeruginosa 4	R	R	S	S	R	R	S	S	S	S	4 (4 0 %)
	P. aeruginosa 5	R	R	S	S	R	R	R	R	S	S	6 (6 0 %)

Key: R= Resistance, I= Intermediate, S= Sensitive

Table 4: Determination of Activity of Tested Antibiotics against Gram-Positive Isolates

S/N	Antibiotics	Activity		
		R	I	S
1	Vancomycin	0	4(20%)	16(80%)
2	Oxacillin	18(90%)	0	2(10%)
3	Cloxacillin	0	2(10%)	18(90%)
4	Penicillin	14(70%)	1(5%)	5(25%)
5	Erythromycin	3(15%)	0	17(85%)
6	Tetracycline	8(40%)	0	12(60%)
7	Chloramphenicol	0	3(15%)	17(85%)
8	Ceftriaxone	1(5%)	2(10%)	17(85%)
9	Amoxicillin	14(70%)	0	6(30%)
10	Gentamycin	1(5%)	1(5%)	18(90%)
11	Ciprofloxacin	0	0	20(100%)
12	Trimethoprim	4(20%)	2(10%)	14(70%)

Table 5: Determination of Activity of Tested Antibiotics against Gram-Negative Isolates

S/N	Antibiotics	Activity		
		R	I	S
1	Septrin	12 (54.55%)	1(4.55%)	7(31.81%)
2	Chloramphenicol	16(72.73%)	0	4(18.18%)
3	Sparfloxacin	4(18.18%)	0	16(72.73%)
4	Ciprofloxacin	0	0	22(100%)
5	Amoxicillin	10(45.45%)	1(4.55%)	9(40.91%)
6	Augmentin	13(59.09%)	2(9.09%)	5(22.73%)
7	Gentamycin	7(31.81%)	0	13(59.09%)
8	Pefloxacin	13(59.09%)	0	7(31.81%)
9	Tarivid	8(36.36%)	1(4.55%)	11(50%)
10	Streptomycin	7(31.81%)	2(9.09%)	11(50%)

Table 6: Antibiogram of Resistant Gram-positive Isolates before and After Plasmid Curing

S/N	Isolates		Antibiotics		
			OXA	PEN	AML
1	*S. aureus 1*	Before	R	R	R
		After	S	S	S
2	*S. aureus 2*	Before	R	R	R
		After	S	S	S
3	*S. aureus 4*	Before	R	R	R
		After	S	S	S
4	*S. pyogenes 1*	Before	R	R	R
		After	S	S	S
5	*S. pyogenes 2*	Before	R	R	R
		After	S	S	S

Key: *OXA: - Oxacillin, PEN:-Penicillin, AML:- Amoxicillin*
S:- Susceptible, R:- Resistant.

Table 8: Antibiogram of MDR Gram Negative Isolates before and After Plasmid Curing

S/N	Isolate		Antibiotics				
			SXT	CH	AM	AU	PEF
1	*P. aeruginosa 3*	Before	R	R	**R**	**R**	S
		After	R	R	**S**	**S**	S
2	*P. aeruginosa 4*	Before	R	R	R	R	S
		After	R	R	S	S	S
3	*K. pneumoniae 4*	Before	R	R	S	R	R
		After	R	R	S	S	S
4	*P. vulgaris 1*	Before	R	R	S	S	R
		After	R	R	S	S	S
5	*E. coli 7*	Before	S	S	S	**R**	**R**
		After	S	S	S	**S**	**S**

Key: STX:- Septrin, CH:- Chloramphenicol, AM:- Amoxicillin, AU:- Augmentin, PEF:- Pefloxacin.
S:- Susceptible, R:-Resistant.

NB: Red colour indicates where no curing activity occurred, and green indicates where curing activity occurred.

5.1 Discussion

The role of microorganisms in impaired healing and enhancement of wound chronicity is quite indispensable. This study was conducted to identify and determine the antibiogram of different bacterial isolates associated with wounds. Consistent with a similar study conducted by Garba *et al* [17], result of this study showed that Gram-negative bacteria were the dominant isolates consisting of 22(52.29%) compared to Gram-positive isolates with 20(47.58%). In contrast to this finding, another study by Rai *et al* [18] reported Gram-positives to be more prevalent in wounds occurring in 61% of the total samples tested. However, another study conducted on wound microbiome, suggested that there is significant dissimilarity in wound etiology with regards to wound/host environment which are among critical issues confounding the efforts to associate specific microbiomes with wound outcomes [19].

Overall, *S. aureus* was found to be the predominant isolate with isolation rate of 26.19%. Similarly, several researchers have identified *Staphylococcus aureus* as the most predominant bacterial pathogen in wounds [9, 19, 17]. This bacterium has long been recognized as one of the important bacteria that cause diseases in humans. Studies have revealed that the presence of *S. aureus* in wound can result in formation of strong biofilm that maintains chronic infection and increased antibiotic resistance, thus impairing the healing of wound [20]. *Staphylococcus aureus* causes clinically relevant infections mostly because of its virulent factors such as coagulase, catalase, clumping-factor A and leucocidines [21].

Trailing *S. aureus* were *Escherichia coli* and *Klebsiella pneumoniae* with occurrence rate of 19.05% and 14.29% respectively. The occurrences of these microorganisms in wounds has been reported in different literatures [11,19,22] and are identified among the leading causes of infection in wounds. Consistent report from Guan *et al* [4] indicated that *E. coli* and *K. pneumoniae* are among the most frequently isolated bacterial species from wounds.

Staphylococcus epidermidis and *Pseudomonas aeruginosa* were having isolation rate of 11.9% each. *S. epidermidis* is by far the best studied member of the coagulase negative staphylococci (CoNS) family and can be isolated from all skin microenvironments, including, dry, moist, subcutaneous and foot region [23]. Some studies have shown that the presence of this bacterium in wounds is beneficial as it induces CD8+ T cells that induce the re-epithelization of the skin after injury, thereby accelerating wound closure [24]. Contrary to its beneficial presence, *S. epidermidis* can play pathogenic role in wound infections as some strains along with other several bacterial species have been reported to associate with chronic infections [25]. *Pseudomonas aeruginosa* produce very destructive virulent factors, responsible for maintaining infection and delay healings in chronic wounds. Similarly, the production of an elastase by *P. aeruginosa* has been associated to its pathogenicity in the wound environment [26].

Other bacteria isolate with lower isolation rate were *Streptococcus pyogenes* with 7.14%, *Proteus vulgaris* with 7.14% and lastly, *Bacillus subtilis* with 2.38%. The presence of these microorganisms in wounds have been reported in studies conducted in India by Mashita *et al* [27] and Nigeria by Shittu *et al* [28] respectively. Infection with *S. pyogenes* causes a wide variety of ailments in humans, including necrotizing fasciitis; mortality is high even with treatment [29]. The bacterium is beta haemolytic and also the agent of scarlet fever and streptococcal toxic shock syndrome. It is also identified among organisms that can cause myonecrosis. Wound infection with *S. pyogenes* may also result in myonecrosis, which is an aggressive, often life-threatening infection that can develop in any open wound [30]. *Proteus vulgaris* is among the most frequently recovered microorganisms from infected wounds. In a similar study conducted by Mordi and Momoh [31] in Benin, Nigeria, *Proteus species* were reported to be the most isolated amongst the Gram negative facultative anaerobic bacilli from wound. Bennett *et al* [32] stated that *Proteus vulgaris* alongside *Proteus mirabilis* accounts for most clinical *Proteus* isolates as they can produce urease and hydrogen sulfide.

Bacillus subtilis, being the isolate with the least occurrence has over time been used in treatment of open wounds. The process employs the administration of sticky dissolvable polyvinyl alcohol (PVA) microparticles containing live *Bacillus subtilis* directly into an open wound where it produces and secrete antimicrobial molecules that are found to antagonize other pathogenic bacteria found in the wound. This approach has demonstrated a remarkable antimicrobial against MRSA *S. aureus* and other bacterial wound pathogens thus, effective in decreasing wound healing time [33]. This concept of combining live secreting bacteria within a supportive delivery system shows great promise as a therapeutic agent for open wounds and other infectious skin disorders. Savistkaya *et al* [34] also stated that the presence of *B. subtilis* in open wound is beneficial as it has demonstrated high antagonistic activity towards causative agents of wound infections such as *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa*.

On accessing the antibiogram of the isolates, a considerable resistance among gram positive isolates was observed towards oxacilin (19%) followed by penicillin (70%) and Amoxicillin (70%) which was also reported in Italy by Kirketerp-Muller *et al* [5]. Similarly, increasing resistance to β-lactam antibiotics among both Gram-positive and Gram-negative bacteria have

been reported in recent years. Fisher and Mobashery [35] also stated the value of the β-lactam antibiotics for the control of bacterial infection has eroded with time as most Gram-positive human pathogens that were routinely susceptible to β-lactam chemotherapy are now not.

On the other hand, the Gram-positive isolates were observed to be significantly susceptible to Ciproxacin (100%), cloxacilin (90%), Gentamycin (90%), Ceftriaxone (85%), erythromycin (85%), Vancomycin (80%). This result was consistent to that of Alhumaid *et al* [36] who reported that highest susceptibility of Gram-positive clinical isolates was seen towards vancomycin, Cloxacillin, and streptomycin.

The most resisted antibiotic among Gram-negatives was Chloramphenicol with 72.73% of the isolates followed by Augmentin (59.09%), Pefloxacin 59.09, Septrin (54.55%) and Amoxicillin with 45.45%, which was also reported in a study conducted in Bahir Dar, Ethopia by Biadglegne *et al* [37] and Mulu *et al* [13]. Similarly, Tersagh *et al* [38] reported significant resistance among Gram-negative isolates against amoxicillin, augmentin, chloramphenicol, pefloxacin and sparfloxacin.

Ciprofloxacin was found to be highly effective with 100% effectiveness against all the Gram-negative isolates followed by sparfloxacin (72.73%), gentamycin (59.9%), tarivid (50%). A consistent report was given by Anejo-Okopi *et al* [39] that most Gram–negative isolates have shown considerable susceptibility to ciprofloxacin, Tarivid (Ofloxacin) and streptomycin among others. The increasing trends of resistance among bacterial isolates towards most conventional antibiotics may be due to massive use of antimicrobials in the area without prescription, empirical treatment option by physician or prolonged use of them.

Among the Gram-positives, *Staphylococcus aureus* and *Streptococcus pyogenes* isolates were found to be the most resistant isolates with some isolates resisting 50% of the tested drugs. This finding concurred with that of other similar studies conducted previously [40, 41]. Gram-negative isolates that showed multiple drug resistance were *P. aeruginosa, E. coli, K. pneumoniae* and *P. vulgaris,* resisting 50 -60% of the tested antibiotics. A similar study conducted by Kabanangi *et al* [42] in Tanzania also reported that most wound isolates of *P aeruginosa, K. pneumoniae* and *E. coli* alongside other Gram-negative wound isolates were multidrug resistant.

On determining the mechanism of antibiotic resistance among the isolates, overnight incubation of the isolates in 10% sodium dodecyl sulphate (SDS) suggested that resistance to oxacillin, penicillin, amoxicillin, augmentin and pefloxacin were plasmid borne. This is finding agreed with the work of Zaman *et al* [16]. Similarly, plasmid mediated resistance against beta lactam antibiotics among both Gram-negative and Gram-positive bacteria have widely been reported over the years and it on the increase as there is rapid spread of these resistance genes among bacteria. Consistent to this finding, report from a study by Ojo *et al* [43] suggested that resistance to beta lactams among most bacteria was plasmid borne. However, Kotb *et al* [44] reported resistance to amoxicillin to be chromosomal in *S. pneumoniae,* suggesting that resistance to beta lactams may also be chromosomal.

Both ciprofloxacin and pefloxacin belongs to the fluoroquinolones group of antibiotics but the Gram-negative isolates have demonstrated a considerable resistance to pefloxacin compared to ciprofloxacin being the most effective of all the tested antibiotics against both Gram-positive and Gram-negative isolates. While pefloxacin has been used as a surrogate marker for quinolone resistance by researchers like Sharma *et al* [45] and Kali *et al* [46], other studies reported susceptibility to ciprofloxacin among isolates resistant to pefloxacin [47]. Reports have also shown that among the fluoroquinolones class, ciprofloxacin is the most potent against gram

negative bacilli (notably, the *Enterobacteriaceae*, such as *E. coli*, *Salmonella* spp., *Shigella* spp. and *Neisseria*) [48].

On the other hand, resistance to septrin and chloramphenicol persisted among the isolates even after plasmid curing, suggesting that it may be chromosomal. Bennett *et al* [32] reported that the main mechanism of resistance to septrin (trimethoprim) and sulfonamides is permeability barrier. Reports have also shown that resistance against trimethoprim (septrin) could result from overproduction of chromosomal dihydropholate reductase (DHFR) caused by promoter mutation [49]. Similarly, Dale *et al* [50] also reported that, a single amino acid substitution in the dhfr gene and altered chromosomally encoded DHFR are responsible for resistance to trimethoprim in *S. aureus* and *S. pneumoniae*. Chromosomal resistance to chloramphenicol has been reported to be mediated by the enzyme chloramphenicol acetyl transferase (CAT) encoded on chromosomal cat gene in *Proteus* spp. [51]. Schwarz *et al* [52] accessed the molecular basis of bacterial resistance to chloramphenicol their result indicated that the resistance may either be chromosomal, plasmid mediated or in some isolates, both depending on the location of the cat genes.

5.2 Conclusion

The outcome of this study revealed that bacteria associated with wound infections encompass both Gram-negative and Gram-positive bacteria in nearly equal proportions, with Gram-negatives having slightly higher isolation rate in the study area. However, this finding may vary with regards to geographical location and time. There is high rate of multidrug resistance among the isolates, and resistance towards β-lactams and pefloxacin among the tested antibiotics are plasmid borne, whereas on the other hand, resistance to trimethoprim (septrin) and chloramphenicol were not plasmid borne, suggesting that the resistance were chromosomal. Continuous surveillance is essential so as to guide appropriate therapy for wound infection and rational use of antimicrobial agents. Similarly, personal hygiene should be maintained by patients to minimize the risk of wound infection. Also, indiscriminate use of antibiotics by patients should be avoided in order to minimize the risk of emergence of multidrug resistant (MDR) pathogen which may be helpful in enhancing wound healing and management.

Lastly, plasmid mediated resistance to antibiotics among bacterial isolates has posed a great threat to modern chemotherapy, it is required therefore, that new strategies to tackle antimicrobial resistance by targeting bacterial plasmids and other transposable elements should be advocated.

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