



Profile and Antimicrobial Susceptibility Pattern of Bacterial Isolates from Infected Wounds of Patients Attending Berekum Holy Family Hospital in Ghana

Isaac Dadzie^{1*}, Nicholas Quansah¹ and Emmanuel Kingsley Dadzie¹

¹*Department of Medical Laboratory Science, School of Allied Health Sciences, University of Cape Coast, Cape Coast, Ghana.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors ID, NQ and EKD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Wound management and care after an infection has been known to present a major concern to healthcare professionals. Its association with increased trauma, discomfort and financial burden to patients bring to bear the need for aggressive intervention. This study sought to investigate the bacterial profile and their antimicrobial susceptibility pattern of infected wounds in patients who reported to Berekum Holy Family Municipal Hospital in Ghana.

Methodology: A total of 150 consenting patients with wound infections of diverse aetiologies were recruited. Wound swabs were aseptically obtained, macroscopically analyzed, gram stained and cultured on differential media. Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion technique.

Results: The prevalence of wound infection was 84.67% with cellulitis (35.43%) accounting for the most cause. Gram-negative bacilli (84.5%) were the most isolated bacteria with *Pseudomonas aeruginosa* (28%) being the most predominant pathogen isolated. Polymicrobial infections were identified in 33 (19.64%) of the infected wounds with *Pseudomonas aeruginosa/Proteus mirabilis*

*Corresponding author: E-mail: idadzie@ucc.edu.gh, iedadzie@gmail.com;

being the most common association (36.4%). The isolated gram-negative organisms were absolutely susceptible (97%-100%) to amikacin. Gentamicin, Ciprofloxacin and Levofloxacin exhibited moderate to higher susceptibility. Gentamicin exhibited maximal susceptibility against the gram-positive organisms with tetracycline being the most resistant antibiotic against the isolated organisms.

Conclusion: With the increased detection of bacterial pathogens causing wound infections and their susceptibility to the tested antibiotics, we recommend that patients with wound infections should immediately report cases to health facilities for early intervention.

Keywords: Wound infection; antimicrobial resistance; pathogens; susceptibility testing.

1. INTRODUCTION

The loss of epithelial continuity as a result of a break in the protective function of the skin, with or without loss of the underlying connective tissue defines a wound [1]. The wound provides suitable conditions conducive for microbial colonization and multiplication culminating in infection. Presentation of patients' wounds can be post-operative, traumatic as a result of an accident, skin burns or chronic due to diabetes, leg and pressure ulcers. The development of wound infection is disheartening to patients due to its associated distress and discomfort.

Wounds, irrespective of the cause may be contaminated with microorganisms that are part of the skin microflora or within the environment and the type and quantity of these microbes vary from one wound to another [2]. Wound infection depends on the virulence of the microorganisms and on the immune competency of the host and it is determined by the presence of clinical signs of infection such as erythema, pain, tenderness, heat, edema, cellulites and abscess [3]. Therefore, wound infection results in active disease that is likely to delay the healing process and cause wound breakdown [4,5].

Wound care constitutes an important part of routine care given by clinicians in resource-rich centers. Sub-Sahara African populace however, continue to rely on traditional methods of caring for wounds. These have resulted in poor healing and increased mortality and morbidity, and subsequent reduction in the function and quality of life. The extended and unregulated use of antimicrobial agents in sub-Sahara Africa and the lack of sterile conditions could be a potent contributor to the resistance of microorganisms associated with wound infections, predisposing to debilitating effects.

Staphylococcus aureus and *Pseudomonas aeruginosa* have been identified to account for

20-40% of wound infection and 5-15% nosocomial infections respectively, with infection mainly following surgery and burns [6]. Studies in other polymicrobial chronic infections suggest that the presence of specific pathogens is more important than that of the bacterial burden [7, 8], contributing to the non-healing outcome in acute and chronic wound infections. These microbes over the years because of sustained use of systemic and topical antimicrobial agents have provided the selective pressure that has caused emergence of antibiotic resistant strains.

In Ghana, antibiotics may be sold without prescription; usage is largely without guidance from healthcare professionals thereby resulting in the emergence of antibiotic resistant bacterial strains [9]. Data representing the susceptibility pattern of bacterial isolates in wound infections to antimicrobials are lacking and hence we sought to investigate the bacterial profile and determine their antimicrobial susceptibility pattern of infected wounds in patients who reported to Berekum Holy Family Municipal Hospital in Ghana.

2. METHODOLOGY

2.1 Study Design

A cross-sectional study was employed to sample wounds of diverse aetiologies from patients who visited the municipal hospital. The wound swab samples from the patients were analyzed.

2.2 Study Area

This study was conducted at Berekum Holy Family Municipal Hospital. The hospital provides a 24-hour Emergency and Therapeutic services; General Out Patient Department, Eye Care, Dental Care, Obstetrics and Gynaecology, General Surgery, Reproductive and Child Health/Family Planning and Counseling. Specialized Clinics such as Diabetes,

Hypertension and Anaemia are also present in addition to a Nursing Training College and Midwifery Training School. The Municipal is one of the twenty-nine (29) administrative districts in the Brong Ahafo Region of Ghana.

2.3 Study Population and Sampling

A total of 150 consenting patients suspected of wound infections were recruited into the study. Patients on antibiotic therapy two (2) weeks prior to the study were excluded. Swabs were aseptically obtained from the sites of wound infection. The specimens were registered and macroscopically examined for their appearances. The swabs were cultured, and smears made on clean slides for Gram-staining techniques. Inoculations of the specimens were done on MacConkey agar and blood agar. The culture plates were incubated aerobically for 24–48 hours before colonial morphologies interpreted. Pure cultures were characterized using morphological appearances on selective and differential media. Biochemical tests were carried out according to standard techniques [10].

2.4 Susceptibility Testing

Susceptibility testing was performed by Kirby-Bauer disk diffusion method. The test organism was uniformly seeded over Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium. The isolates were then incubated at 37°C for 16–18 hours. Interpretation was done according to the recommendations of the National Committee for Clinical Laboratory Standards [11]. Antibiotics tested against Gram-positive cocci were cotrimoxazole (25 µg), erythromycin (10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), meropenem (10 µg), vancomycin (30 µg), penicillin (10µg), linezolid (30 µg), lincomycin (10 µg), flucloxacillin (5 µg), tetracycline (10 µg), cefuroxime (30 µg), augmentin (30 µg), and gentamicin (10 µg). Gram-negative rods antibiotics were gentamicin (10 µg), cefotaxime (30 µg), ceftizoxime (30 µg), ciprofloxacin (5 µg), chloramphenicol (10 µg), amikacin (10 µg), levofloxacin (5 µg), cotrimoxazole (25 µg), piperacillin-tazobactam (40 µg), ofloxacin (5 µg), tetracycline (10 µg), and ampicillin (10 µg).

3. RESULTS

The study recruited one hundred and fifty consenting (150) patients with various types of

wound infections. Of these, 64 were males and 86 were females. A total of 127 samples (84.67%) yielded significant bacterial growth indicative of wound infection, whereas 23 samples yielded insignificant growth. Fig. 1 summarizes the prevalence of significant bacterial growth in different types of wound infections. Cellulitis (45, 35.43%) recorded the highest significant bacterial growth followed by surgical site infection (40, 31.50%) with abscess (6, 4.72%) recording the least significant bacterial growth.

Pseudomonas aeruginosa (28%) and *Escherichia coli* (17.9%) were the most isolated gram-negative bacilli, while *Acinobacter spp*, *Klebsiella spp*, *Providencia spp*, *Morganella morganii* and *Serratia marcescens* were the least isolated. *Staphylococcus aureus* (12.5%) and coagulase negative *Staphylococcus* (3.0%) were the only Gram-positive organisms isolated from the wound swabs. Bacterial isolates were recorded in all surgical site infections whereas abscess recorded the least isolated bacteria (Table 1).

Table 2 shows the prevalence of wounds infected with more than one bacteria. Single species (80.36%) were the most isolated bacteria from each sample. Polymicrobial infections were identified in 33 (19.64%) of the infected wounds and was mainly constituted by two species; three species were found to be 6%. *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus* were the most predominant isolated species culminating in polymicrobial infections with *Pseudomonas aeruginosa/Proteus mirabilis* being the most common association (36.4%).

The isolated bacteria exhibited a high susceptibility to the tested antibiotics with susceptibility levels ranging from 50% - 100%. Almost all the isolated organisms were absolutely susceptible (97%-100%) to amikacin. The bacterial isolates exhibited moderate to higher susceptibility (50%-100%) to gentamicin, ciprofloxacin and levofloxacin. Co-trimoxazole, ampicillin and tetracycline were absolutely resisted by the isolated organisms. *Proteus mirabilis* showed a higher susceptibility (52.6%-100%) to most of the antibiotics than *Escherichia coli* (26.6%-97%) and *Pseudomonas aeruginosa* (17.0%-97.8%) (Table 3).

Table 4 highlights the antimicrobial susceptibility pattern of *Staphylococcus aureus* and coagulase

negative *Staphylococcus* (CoNS). *Staphylococcus aureus* and CoNS exhibited maximal susceptibility to gentamicin (90.4% and 100% respectively), followed by flucloxacillin and ciprofloxacin. *Staphylococcus aureus* was highly resistant (4.7%) to tetracycline, ampicillin, augmentin, meropenem and penicillin, whereas CoNS was absolutely resistant to tetracycline.

4. DISCUSSION

Wound management and care after an infection present a major concern to healthcare

professionals. Its association with increased trauma, discomfort and financial burden to patients bring to bear the need for aggressive intervention. We sought to investigate the bacterial profile and determine their antimicrobial susceptibility pattern of infected wounds in patients who reported to Berekum Holy Family Municipal Hospital. Our findings revealed a total significant bacterial growth prevalence of 84.67% in the entire study population with wound infections. This high prevalence is consistent with a study done by Pondei et al. [1] that involved 101 patients and similar studies by Wariso and

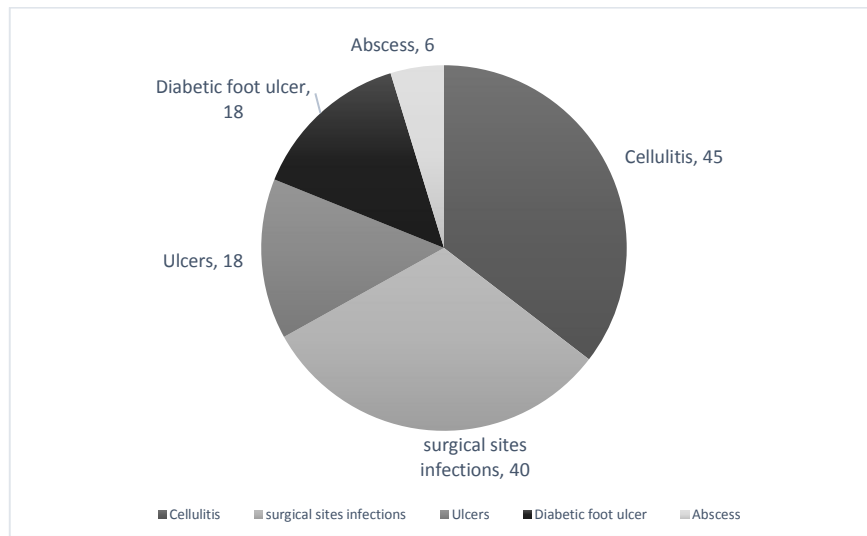


Fig. 1. Prevalence of significant bacterial growth in different types of wound infections

Table 1. Distribution of bacterial isolates stratified according to the type of wound

Bacterial isolates	Cellulitis N (%)	Surgical site infection N (%)	Diabetic foot Ulcer N (%)	Ulcers N (%)	Abscess N (%)	Total (%)
<i>Pseudomonas aeruginosa</i>	19(31.1)	10(20.0)	8(33.3)	8(32.0)	2(25.0)	47(28.0)
<i>Proteus mirabilis</i>	9(14.7)	2(4.0)	4(16.7)	3(12.0)	1(17.5)	19(11.3)
<i>Proteus vulgaris</i>	6(9.8)	4(8.0)	0(0.0)	1(4.0)	2(25.0)	13(7.7)
<i>Escherichia coli</i>	10(16.4)	13(26.0)	4(16.7)	2(8.0)	1(17.5)	30(17.9)
<i>Staphylococcus aureus</i>	9(14.7)	5(10.0)	3(12.5)	3(12.0)	1(17.5)	21(12.5)
<i>Coagulase Negative Staphylococcus</i>	1(1.6)	1(2.0)	1(4.2)	2(8.0)	0(0.0)	5(3.0)
<i>Enterobacter spp</i>	3(4.9)	5(10.0)	2(8.3)	3(12.0)	0(0.0)	13(7.7)
<i>Citrobacter spp</i>	3(4.9)	4(8.0)	1(4.16)	1(4.0)	1(17.5)	10(5.9)
<i>Acinobacter spp</i>	0(0.0)	1(2.0)	0(0.0)	1(4.0)	0(0.0)	2(1.2)
<i>Klebsiella spp</i>	0(0.0)	1(2.0)	1(4.16)	0(0.0)	0(0.0)	2(1.2)
<i>Providencia spp</i>	0(0.0)	2(4.0)	0(0.0)	0(0.0)	0(0.0)	2(1.2)
<i>Morganella morganii</i>	1(1.6)	1(2.0)	0(0.0)	1(4.0)	0(0.0)	3(1.8)
<i>Serratia marcescens</i>	0(0.0)	1(2.0)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
Total	61(36.3)	50(29.8)	24(14.3)	25(14.9)	8(4.8)	168(100)

Table 2. Prevalence of bacterial associations in infected wounds

Bacterial isolates wounds	Cellulitis	Surgical site	Ulcers	Diabetic foot ulcer	Abscess	Total
<i>P. aeruginosa</i> + <i>E. coli</i>	2	1	0	0	0	3(9.1)
<i>P. aeruginosa</i> + <i>Enterobacter</i> spp	0	1	0	0	0	1(3.0)
<i>P. aeruginosa</i> + <i>P. mirabilis</i>	4	1	4	2	1	12(36.4)
<i>P. aeruginosa</i> + <i>E. coli</i> + <i>P. mirabilis</i>	1	0	0	0	0	1(3.0)
<i>P. aeruginosa</i> + <i>S. aureus</i>	2	0	0	0	1	3(9.1)
<i>S. marcescens</i> + <i>Klebsiella</i> spp + <i>P. aeruginosa</i>	0	1	0	0	0	1(3.0)
<i>P. aeruginosa</i> + <i>Klebsiella</i> spp	0	0	0	1	0	1(3.0)
<i>P. aeruginosa</i> + <i>Citrobacter</i> spp	1	0	0	0	0	1(3.0)
<i>Pr. mirabilis</i> + <i>Citrobacter</i> spp	0	1	0	0	0	1(3.0)
<i>P. aeruginosa</i> + <i>Pr. Vulgaris</i>	0	1	0	0	0	1(3.0)
<i>M. morgani</i> + <i>Citrobacter</i> spp	0	0	0	1	0	1(3.0)
<i>E. coli</i> + <i>Enterobacter</i> spp	1	0	1	0	0	2(6.1)
<i>P. vulgaris</i> + <i>S. aureus</i>	1	0	0	0	0	1(3.0)
<i>S. aureus</i> + <i>Citrobacter</i> spp	0	0	0	1	0	1(3.0)
<i>Providencia</i> spp + <i>E. coli</i>	0	1	0	0	0	1(3.0)
<i>P. mirabilis</i> + <i>E. coli</i>	0	0	0	1	0	1(3.0)
<i>P. mirabilis</i> + <i>Citrobacter</i> spp	0	1	0	0	0	1(3.0)
Total	12(36.4)	8(24.2)	5(15.2)	6(18.2)	2(6.0)	33(100)

Table 3. Antimicrobial susceptibility pattern of isolated gram-negative microorganisms

Antibiotics	<i>Pseudomonas</i> N=47	<i>E. coli</i> N= 30	<i>P. mirabilis</i> N= 19	<i>P. vulgaris</i> N=13	<i>Enterobacter</i> N=13	<i>Citrobacter</i> N=10	<i>M. morganii</i> N=3	<i>Acinobacter</i> N=2	<i>Klebsiella</i> N=2	<i>S. marcescens</i> N=1	<i>Providencia</i> N=2
Ampicillin.	1(2.1%)	1(3.3%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Amikacin	46(97.8%)	29(97.0%)	19(100%)	13(100%)	13(100%)	10(100%)	3(100%)	2(100%)	2(100%)	1(100%)	2(100%)
Gentamicin	24(51.0%)	21(70%)	18(94.7%)	11(58%)	8(62%)	8(80%)	2(67%)	0(0.0)	1(50%)	1(100%)	2(100%)
Ciprofloxacin	35(74.4%)	19(63.3%)	17(89.5%)	12(92.2%)	9(69%)	7(70%)	1(33.3%)	1(50%)	1(50%)	1(100%)	0(0.0)
Levofloxacin	35(74.4%)	21(70%)	19(100%)	12(92.2%)	9(69%)	9(90%)	1(33.3%)	1(50%)	2(100%)	1(100%)	0(0.0)
Cotrimoxazole	0(0.0)	1(3.3%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50%)	0(0.0)	0(0.0)
Linezolid	25(53.2%)	17(56.7%)	17(89.5%)	11(58%)	10(77%)	7(70%)	1(33.3%)	0(0.0)	1(50%)	1(100%)	1(50%)
Ofloxacin	25(53.2%)	12(40%)	16(84.2%)	9(69%)	8(62%)	8(80%)	1(33.3%)	0(0.0)	1(50%)	1(100%)	0(0.0)
Chloramphenicol	9(19.1%)	8(26.6%)	10(52.6%)	4(31%)	5(38%)	6(60%)	1(33.3%)	0(0.0)	1(50%)	1(100%)	0(0.0)
Cefotaxime	9(19.1%)	12(40%)	16(84.2%)	10(77%)	4(31%)	6(60%)	1(33.3%)	0(0.0)	1(50%)	1(100%)	0(0.0)
Ceftizoxime	8(17.0)	13(43.3%)	16(84.2%)	10(77%)	4(31%)	7(70%)	1(33.3%)	0(0.0)	1(50%)	1(100%)	0(0.0)
Tetracycline	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table 4. Antimicrobial susceptibility pattern of *Staphylococcus aureus* and Coagulase Negative *Staphylococcus* (CoNS)

Antibiotics	<i>Staphylococcus aureus</i> , N=21	CoNS, N=5
Ampicillin	1(4.7%)	1(20%)
Cotrimoxazole	4(19.0%)	1(20%)
Erythromycin	12(57.1%)	3(60%)
Cefuroxime	16(76.2%)	3(60%)
Flucloxacilin	15(71.4%)	4(80%)
Gentamicin	19(90.4%)	5(100%)
Ciprofloxacin	16(76.2%)	3(60%)
Augmentin	1(4.7%)	1(20%)
Vancomycin	13(62%)	3(60%)
Meropenem	1(4.7%)	1(20%)
Penicillin	1(4.7%)	1(20%)
Linezolid	6(28.6%)	1(20%)
Lincomycin	2(9.5%)	1(20%)
Tetracycline	1(4.7%)	0(0%)

Taiwo et al. [12,13]. A retrospective study by Azene and Beyene [14] reported a prevalence of 70.5% which is relatively lower to that of our study.

This study reported cellulitis (35.43%) as the major kind of wound culminating in infection followed by surgery (31.50%), with abscess (4.72%) accounting for the least cause of wound infections. Zarrin et al., [15] reported diabetic foot ulcer (33.0%) to be the most predominant wound with bacterial pathogens followed by abscess (29.6%). Differences in the demographics and geographical locations may account for these variations as the participants in our study were mostly engaged in farming activities. It is evident that the farmers within the community and beyond do not strictly adhere to the use of personal protective gears and hence have high probability of sustaining injuries. Improper first aid and their reluctance in reporting to health facilities enhance contamination and multiplication of the bacterial pathogens.

Majority of the wounds were colonized with a single bacterial species with Gram-negative bacilli yielding the highest prevalence of 84.5% and *Staphylococcus aureus* (12.5%) being the most isolated Gram-positive organism. *Pseudomonas aeruginosa* (28%) was the most isolated gram-negative organism which is consistent with other studies [16,17], and the most predominant isolated microorganism, similar to that of Pondei et al. [1] but in contrast to other studies [4,18,19] which reported *Staphylococcus aureus* as the most common isolate. Data presenting infections caused by these two microorganisms are well documented. They produce very detrimental virulence factors

responsible for maintaining infection and delaying the healing process. Virulence factors such as coagulase, catalase, clumping-factor A and leucocidines produced by *Staphylococcus aureus* causes clinically relevant infections [20] whereas the production of elastase by *Pseudomonas aeruginosa* has been associated to its pathogenicity in the wound environment [21].

With regards to infections, bacteria that have evolved in thriving in similar conditions are mostly found to occupy the same milieu. It was observed that polymicrobial infections were identified in 19.64% of the infected wounds which was relatively lower compared to the study by Bessa et al. [4] which reported 27.2%. They were mainly constituted by two species; three species were found to be 6%. Species interactions result in synergy that enhances survival and thereby hinder eradication of infection. *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus* were the most predominant isolated species culminating in polymicrobial infections with *Pseudomonas aeruginosa/Proteus mirabilis* being the most common association (36.4%).

The use of appropriate systemic antibiotics in therapy is still recommended where there is clear evidence of infection despite concerns about increase bacterial resistant [3,22]. Almost all the isolated organisms were susceptible (97%-100%) to amikacin. Gentamicin, ciprofloxacin and Levofloxacin exhibited moderate to higher susceptibility (50%-100%) against the isolated organisms while co-trimoxazole, ampicillin and tetracycline were absolutely resisted. Gentamicin exhibited maximal susceptibility against

Staphylococcus aureus (90.4%) and coagulase negative *Staphylococcus* (100%). This is in agreement to that of Mama et al. [23] which reported amikacin, levofloxacin, ciprofloxacin and gentamicin to be the most effective antibiotic to gram-negative isolates while ampicillin and tetracycline were the least effective. Although aggressive antibiotic treatment may be necessary to reduce wound infections, this should be done with caution as microorganisms have the ability to establish themselves and proliferate as a biofilm which are often considered to be a further complication that has a significant contribution to the lack of successful antibiotic treatment.

5. CONCLUSION

With cellulitis and surgery culminating in wound infections, it is expedient that resources such as personal protective gears, adequate surgical tools must be available to help eradicate or minimize the prevalence of pathogenic contaminated wounds. With the increased detection of bacterial pathogens causing wound infections and their increased susceptibility to the tested antibiotics, we recommend that patients with wound infections should immediately report cases to the health facilities for early intervention.

CONSENT

As per international standard written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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