



Original Research Article

Isolation and sensitivity pattern of bacterial isolates of wound infections from patients of Federal Medical Centre, Umuahia, Abia State

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ABSTRACT

Purulent specimens were aseptically collected using swab sticks from 56 patients with different wound infections at the Federal Medical Centre (FMC), Umuahia, Abia State. The wound specimens comprising burns (8), post-operative (12), trauma (11), abscesses (14) and boils (11) were subjected to bacteriological analyses using standard methods. A total of 78 isolates were recovered from the specimens giving rise to two gram positive bacteria (*Staphylococcus aureus* and *Streptococcus* species) and five gram negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella* species, *Enterobacter* species, *Escherichia coli* and *Proteus* species). *S. aureus* was the most predominant microorganism constituting 22% of the total isolates recovered from these wound types while wounds from burns gave the highest number of isolates (21). Male patients were found to have more wound specimens (35) and isolates (67.9%) than the female gender (21, 32.1%). *Streptococcus* species was most sensitive to Gentamycin (21.6) followed by *Staphylococcus aureus* and to Gentamycin too, however, the two pathogens were completely resistant to Cloxacillin and Augmentin, respectively and in addition, *S. aureus* was also completely resistant to Tetracycline and Cotrimoxazole. *Enterobacter* species was most sensitive (among the gram negative bacteria) to Ceftazidime antibiotic (26.6), but showed 100% resistance to Erythromycin and Cotrimoxazole respectively. *P. aeruginosa* was completely resistant to five antibiotics: Cloxacillin, Ceftriaxone, Erythromycin, Cephalaxin and Clavulante and this was the highest resistance recorded among the Gram negative isolates. The bacterial pathogens isolated from the wound infections analyzed here are *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella* species, *Enterobacter* species, *Streptococcus* species, *Escherichia coli* and *Proteus* species in that order. Both Gram positive and negative bacteria were implicated with the latter group being more in occurrence. The high level of resistance to antibiotics recorded here is a cause for concern in wound therapy and management.

Keywords

Antibiotics,
Bacterial
pathogens,
Infections,
Sensitivity
profile,
Wounds

Introduction

Wound is a disruption in the continuity of

soft parts of the structures (Singleton and

Sainsbury, 1978; Torpy *et al.*, 2005), or a breach in the skin and the exposure of subcutaneous tissues following loss of skin integrity which provides a moist, warm, and conducive environment that is conducive to microbial colonization and proliferation (Bowler *et al.*, 2001; Shittu *et al.*, 2002). Development of wound infection depends on the interplay of factors. The breaking of the host protection layer- the skin, and thus disturbing the protective functions of the layer, will induce many cell types into wound to initiate host response (Collier, 2003). Infection of the wound is the successful invasion, and proliferation by one or more organisms anywhere within the body's sterile tissues, sometimes resulting in pus formation (Calvin, 1998). Wounds can be classified as accidental, pathological or post-operative. Whatever the nature of the wound, infection is the attachment of microorganisms to host cells and they proliferate, colonize and become better placed to cause damage to the host tissues.

A wound can be considered infected if purulent material is observed without confirmation of a positive culture. The numbers of contaminants may not persist but specifically grow and divide and may become established, causing wound colonization or infection. Infection in a wound delays healing and may cause wound breakdown, herniation of the wound and complete wound dehiscence (Alexander, 1994; Bowler *et al.*, 2001). In spite of technological advances that have been made in surgery and wound management, wound infection have been regarded as the most common nosocomial infection especially in patients undergoing surgery and are associated with increased morbidity and mortality (Dionigi *et al.*, 2001). It is an important cause of illness resulting in a prolongation of hospital stay; increased trauma care, treatment costs, and general wound management practices become more

resource demanding (Akinjogunla *et al.*, 2009). The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by methicillin-resistant *S. aureus*, polymicrobial flora and by fungi (Yah *et al.*, 2004).

The knowledge of the causative agents of wound infections has therefore proved to be helpful in the selection of empiric antimicrobial therapy and on the infection control measures in health institutions. Wound infections can be caused by different groups of microorganisms like bacteria, fungi and protozoa (Bowler, 1998), but in most cases, infection does not develop because innate host defenses are quite efficient in the elimination of contaminants. A complex interplay between host, microbial and other factors (e.g. surgical factors in case of surgical wound) ultimately determines the prevention or establishment of a wound infection.

This research work carried out in 2013 was aimed at isolating and determining the antibiotic sensitivity pattern of pathogens associated with wound infections of patients attending Federal Medical Centre (FMC), Umuahia, Abia State.

Materials and Methods

Sample collection

Wound samples were collected from the 56 patients on different days at the microbiology laboratory of Federal Medical Centre, Umuahia, Abia State. The wound types are post-operative wound, burns, trauma wound, abscesses and boils. All samples were transferred to the microbiology laboratory of Michael Okpara University of Agriculture, Umudike for analysis.

Isolates recovery

The wound swabs were streaked onto freshly prepared Nutrient agar (Antek, UK), Blood agar (Antek, UK) and MacConkey agar (Fluka Biochemika, Germany) and incubated at 37°C for 24 hours. After the incubation, the bacterial colonies that grew on the media were picked and purified by sub culturing onto fresh nutrient agar, blood agar and MacConkey agar plates using streak plate's technique. Isolates that grew on the plates were transferred onto nutrient agar slants with proper labeling. These slants were stored in the refrigerator and were used for identification and characterization (Cheesbrough, 2004, 2005).

Identification of bacterial isolates

The isolates were subjected to further identification and characterization based on Standard Microbiological Methods by Monica Cheesbrough (2004, 2005).

Preparation of turbidity standard equivalent to Mcfarland 0.5

One percent (1%) v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. 1% of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml of distilled water. 0.6ml of Barium chloride solution was added to 99.4ml of the sulphuric acid solution and mixed properly. The solution was preserved in the fridge.

Antibiotics sensitivity test

In vitro susceptibility of the organisms to the antibiotics was determined using Bauer disk-diffusion technique (Bauer *et al.*, 1996). Commercially antibiotics discs containing the different concentrations (μg)

were used. A total of 8 antibiotics (Abtek Biologicals Limited, UK) were used for the study (for Gram positive and negative bacteria respectively). A colony of the isolates was streaked on the entire surface of prepared Mueller Hilton agar plate (Scharlau Chemie, Spain) and the antibiotic disc was impregnated into the plate using a sterilized forceps. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured using a caliper. The interpretation of the measurement as sensitive, intermediate and resistant was made according to the standard zone size interpretative manual by Cheesbrough (2004).

Results and Discussion

From the results, a total of 46 isolates (58.9%) were recovered from wound types that required incision and drainage (I.D) to remove the exudates and clean the underlying tissue (incised boils, abscesses and post-operative wounds). These isolates were found to be two gram positive bacteria (*Staphylococcus aureus*, *Streptococcus* species) and five gram negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella* species, *Enterobacter* species, *Escherichia coli* and *Proteus* species). *S. aureus* was the most predominant microorganism constituting 22% of the total isolates recovered from these wound types. There were variations in the occurrences and distributions of bacterial isolates.

Wounds from burns gave the highest number of isolates (21) but incidentally had the least number of clinical specimens collected (8) (Table 1). This high number of isolates may be associated with wide range of the skin affected by the burns which offered larger surface area of contamination from the hosts' skin flora and from the outside environment. Most wound infections

are contaminated by the patients' own endogenous flora, which are present on the skin, mucous membrane, or hollow viscera. The traditional microbial concentration quoted as being highly associated with surgical site infections (SSI) is that of bacterial counts higher than 10,000 per gram of tissue (or in the case of burned sites, organisms per cm² of wound) (Krizek and Robson, 1975). However, different microbes can exist in polymicrobial communities especially in the margins of wounds and in chronic wounds. The infecting microorganism may belong to aerobic as well as anaerobic group *P. aeruginosa* had highest occurrence level (9%; from burn wounds) and in all the wound specimens compared with other isolates (Table 1).

The least number of isolates was recovered from trauma wounds are 11. The wounding agents range from nail pricks to door slams on fingers and motorcycle accidents. Trauma is often associated with the development of local or systemic infection and the situation to which injury or trauma occur as well as the location of the injury may be predictive of the number and types of pathogens found in the wound. Infecting microorganisms may be derived either from an exogenous source (i.e. water-borne from water-related injury or microorganisms from soil in soil-contaminated injury), or the endogenous microflora of the patient (File, 1995).

Male patients were found to have more wound specimens (35) and isolates (67.9%) than the female gender (21, 32.1%: Table 2). This might be due to the risk taking nature of the males both as youths and adults especially in functioning as the breadwinners of the families. The males could be more adventurous and endearing in physical activities thus getting more exposed to the various agents of wounds and their

infections than their female counterparts.

S. aureus was the commonest pathogen isolated from the specimens analyzed (28.2%; Table 3), followed by *P. aeruginosa* (17.9%) while *Proteus* species was the lowest (7.7%). *S. aureus* is both a human skin commensal and a frequent cause of clinically important infections including boils, styes, pustules, impetigo, infections of wounds (cross infections), osteomyelitis, (Lowy, 1998; Cheesbrough, 2005). This would explain its widest spread presence more than all the isolates in all the wound specimens collected. The second position occupied by *P. aeruginosa* (17.9%) in the occurrence can be explained on the ground that it is a free living bacterium and can be found in the bowels of 5% healthy persons (Mckane and Kandel, 1996), water, soil and sewage and is frequently found in moist environments in hospitals (sinks, cleaning buckets, drains, humidifiers etc). Because of this, many infections with *P. aeruginosa* are opportunistic hospital-acquired, affecting those already in poor health and immunosuppressed.

Streptococcus species was most sensitive to Gentamycin (21.6mm) followed by *Staphylococcus aureus* and to Gentamycin too (21mm; Table 4) and this implies that the two antibiotic would offer good options to the two pathogens in therapy. However, the two pathogens were completely resistant to Cloxacillin and Augmentin, respectively, and in addition, *S. aureus* was also completely resistant to Tetracycline and Cotrimoxazole. The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by methicillin-resistant *Staphylococcus aureus*, polymicrobial flora and by fungi (Yah *et al.*, 2004).

The sensitivity profile of the gram negative bacteria showed that *Enterobacter* species was most sensitive to Ceftazidime antibiotic (26.6mm; Table 5). Although this pathogen showed 100% resistance to Erythromycin and Cotrimoxazole respectively, *P. aeruginosa* was completely resistant to five antibiotics: Cloxacillin, Ceftriaxone, Erythromycin, Cephalaxin and Clavulante and this was the highest resistance recorded among the Gram negative isolates. Numerous reports have demonstrated that hospital environment surfaces are sources of antibiotic-resistant bacteria such as *Pseudomonas aeruginosa*, *Enterobacter* spp., *Klebsiella* spp. (Gang *et al.*, 1999). The microbiological analysis reveals that *S. aureus* is the leading etiologic agent of wound infections, followed by *Pseudomonas* species. This is similar to reports in Nigeria (Emele *et al.*, 1999), India (Basak *et al.*, 1992), Thailand (Tranet *et al.*, 1998) and Japan (Mashita *et al.*, 2000). This was however in contrast to the findings of Stainer *et al.* (2003) which showed that *Pseudomonas* was the commonest pathogen isolated. *Pseudomonas aeruginosa* in this study was resistant to third generation cephalosporins (ceftazidime) (Table 5). A primary cause of drug resistance in gram-negative bacteria is their ability to generate extended-spectrum β -lactamase, that can inactivate penicillin and cephalosporins, which are necessary in treatment of infections. Nowadays, *P. aeruginosa* other gram-negative bacteria (*Enterobacter*, *Klebsiella*) are responsible for nosocomial infections in wounds (Trilla, 1994). Colonized patients and staff are a major source of cross-

contamination of other patients.

In conclusion, contracting wound infection remains an on-going problem. The main culprits of wound infections are *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella* species, *Enterobacter* species, *Streptococcus* species, *Escherichia coli* and *Proteus* species in that order. Both Gram positive and negative bacteria were implicated in wound contamination with the latter group being more in number. The high level of resistance to antibiotics seen here is a cause for concern in wound therapy and management.

Recommendation

Recognition of infectious persons is necessary to prevent cross-transmission of infection. Other factors such as failure to prevent cross-transmission in hospital and the use of broad spectrum antibiotics are responsible for increased drug resistance, bacterial colonization and some infections in wounds. In addition, other contributing factors are long periods of hospitalization, procedures for hospital infection control, and the number of patients. Therefore, it is necessary to implement a comprehensive education campaign for all health workers and establish more effective infection control practices and policies in wound units. Results reported in recent studies have determined that being exposed to heavy antibiotic use, high-density patient population that is in frequent contact with health care staff, and the attendant risk of cross-infection are important factors in the issue of antibiotic resistance.

Table.1 Occurrence and distribution of bacterial isolates in wounds (%)

Wound type	No of samples (%)	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella</i> species	<i>Escherichia coli</i>	<i>Enterobacter</i> species	<i>Proteus</i> species	<i>Streptococcus</i> species	Total no of isolates
Post-operatives	12 (21.40)	5	3	4	2	2	1	2	19
Burns	8 (14.30)	4	9	NI	2	3	1	2	21
Trauma	11 (19.60)	6	NI	2	NI	NI	2	1	11
Abscesses	14 (25.00)	3	2	3	2	1	1	1	13
Boils	11 (19.60)	4	NI	2	2	3	1	2	14
Total	56 (100)	22	14	11	8	9	6	8	78

Table.2 Occurrence of bacterial isolates in relation to sex

Sex	No of samples	No of isolates	% of isolates
Males	35	53	67.90
Females	21	25	32.10
Total	56	78	100.00

Table.3 Percentage occurrence of bacterial isolates in wounds

Isolates	Total no isolated	% of isolates
<i>Staphylococcus aureus</i>	22	28.2
<i>Pseudomonas aeruginosa</i>	14	17.9
<i>Streptococcus</i> species	8	10.3
<i>Klebsiella</i> species	11	14.1
<i>Enterobacter</i> species	9	11.5
<i>Escherichia coli</i>	8	10.3
<i>Proteus</i> species	6	7.7
Total	78	100

Table.4 Antibiotic sensitivity pattern of Gram positive bacterial isolates (mm)

Isolates	Cloxacillin (5µg)	Streptomycin (10µg)	Gentamycin (10µg)	Tetracyclin (10µg)	Erythromycin (5µg)	Chloramphenicol (30µg)	Cotrimoxazole (25µg)	Augmentin (30µg)
<i>Staphylococcus aureus</i>	R	18.3	21	R	20.6	18	R	R
<i>Streptococcus</i> species	R	18.4	21.6	20	20.3	17.5	I	R

KEY: R = Resistant; I = Intermediate

Interpretative Refrence Range

CODE	SENSITIVE	INTERMEDIATE	RESISTANT
Cloxacillin	≥18	11 – 15	≤10
Streptomycin	≥17	12 – 16	≤11
Gentamycin	≥15	13 – 14	≤12
Tetracycline	≥19	12 – 18	≤11
Erythromycin	≥23	14 – 22	≤13
Chloromphenicol	≥14	10 – 13	≤9
Cotrimoxazole	≥22	14 – 21	≤13
Augmentin	≥15	13 – 14	≤13

Table.5 Antibiotic sensitivity pattern of Gram-negative isolates (mm)

Isolates	Ceftazidime (10µg)	Cloxacillin (5µg)	Gentamycin (10µg)	Ceftriaxone (10µg)	Erythromycin (5µg)	Cephalaxin (5µg)	Cotrimoxazole (25µg)	Clavulanate (10µg)
<i>Pseudomonas aeruginosa</i>	22.9	R	15.8	R	R	R	17.7	R
<i>Klebsiella</i> species	23.2	14.6	15.7	R	24	R	R	R
<i>Enterobacter</i> species	26.6	15.1	25	I	R	23	R	24
<i>Escherichia coli</i>	18.7	16.2	17.0	R	24.6	24	R	22
<i>Proteus</i> species	18.8	16.4	16.7	R	24	23.8	18	R

KEY: R = Resistant, I = Intermediate.

Interpretative reference range:

CODE	SENSITIVE	INTERMEDIATE	RESISTANT
Ceftazidime	≥18	11 – 15	≤10
Erythromycin	≥23	14 – 22	≤13
Ceftriaxone	≥23	15 – 22	≤14
Cephalaxin	≥22	14 – 21	≤13
Cotrimoxazole	≥17	15 – 16	≤14
Clavulanate	≥21	16 – 20	≤15
Cloxacillin	≥14	10 – 13	≤9
Gentamycin	≥15	13 – 14	≤12

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