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Research Paper

Antibiotic Susceptibility of Bacterial Isolates from Post and Non Operative Wound Sepsis in Patients attending Federal Medical Centre Keffi

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It is a known fact that wound sepsis in rural areas than in cities due to general life style. Thus, this research was to establish likely bacterial isolates from post and non operative wound sepsis in patients attending Federal Medical Centre Keffi and to ascertain the susceptibility of such isolates to conventional antimicrobials and some newly developed ones. A total of 100 swab samples were aseptically collected from post and non operative wound septic patients. Investigations were based on cultural, microscopic identification and characterization of bacterial isolates. *Staphylococcus aureus* had the highest percentage frequency of 44(58.67%), *Pseudomonas aeruginosa* 20(26.67%) while *Escherichia coli* recorded 11(14.67%) (*S.aureus* > *P. aeroginosa* > *E.coli*). Male patients accounted for 53(77.94%) infection rate while female patients accounted for 15(22.06%). *S. aureus* was most susceptible to cloxacillin (52.27%) than other antibiotics used. Ciprofloxacin exhibited highest efficacy (65.00%) against *P. aeruginosa* while *E. coli* showed most susceptibility (30%) to cloxacillin among the antibiotics tested. High occurrence of *S.aureus* could be linked to the fact that it is a commensal of the skin as opposed to *P. aeruginosa* and *E. coli*. Males were affected than females probably because they are being exposed to farm work, fishing and other hazardous activities than their counterparts. For effective antibiotic theraphy, the etiologic agent involved in a particular wound sepsis should be determined and antibiogram conducted on the identified bacterial agent to reduce emergence of multidrug resistant phenomenon.

Keywords: Wound sepsis, hygiene, bacteria, antibiotics susceptibility

INTRODUCTION

Wound sepsis has been an integral part of life found mostly in the rural parts of the country due to the low level of personal hygiene of the people in their socio-economic life style. Moreover, farmers that go to farm bare-footed whose wounds could be vulnerable to invasion of micro-organisms notably bacteria are at high risk, also post-operative wound infections have been found to pose a global problem in the field of surgery (Mengesha *et al.*, 2014). It may occur as a primary wound infections due to some other complications (Zuarez–Estoan *et al.,* 2017). In fact, most post-operative wound infection are hospital acquired (Nosocomial infection) as reported by Mehta *et al. (2014*). Lopiso *et al.* (2014) carried out a microbial analysis on pus samples obtained from 194 patients with clinical diagnosis of post-surgical wound infection of Hawassa Teaching and

Referral Hospital, from November 2010 to March 2011. They reported isolation rate of aerobic bacteria obtained to be 138 (71.1%) and *Staphylococcus aureus* was the most frequent isolate (37.3%); followed by *E. coli* (25.4%), *Klebsiella* species (13.6%), *Proteus* (10.2%) *Pseudomonas aeruginosa* (10.2%) and coagulase negative *Staphylococci* (3.4%).Single and multiple anti-microbial resistances were observed in 6.8% and 93.2% of isolates respectively. No bacterial isolates was found to be sensitive to all antibiotics commonly use. The global epidemiology of surgical site infection (SSI) has not been well described due to lack of standard criteria for diagnosis. The global prevalence of nosocomial infections was estimated at 3-21% of which, wound infections accounted for 5-34% (Samuel *et al.,* 2010), while Khan *et al.(*2017) reported nosocomial infections to account for 7% in developed and 10% in developing countries.

Patients with Surgical Site Infection (SSI) had a significantly extended hospital stay incurred higher costs and increased risk of mortality compared to those without these infections (Suchitra and Lakshnideri, 2009). Bacterial factors such as inoculums size, virulence, and invasive capability as well as immunological and physiological state of the host, influence occurrence of surgical wound infection (Masaadeh and Jaran, 2009). Burns provides a suitable site for bacteria multiplication and is more persistent richer sources of infection than surgical wounds (Alebachew *et al*., 2012) mainly because of the larger area involved and longer duration of patient stay in the hospital (Ashwani *et al*., 2011).The infection of burn wounds with multiple organisms, with superadded problem of drug resistance, illustrates the need for a drug policy by the hospitals for burn patient. The resistance to antimicrobials is largely as a result of various resistant factors borne by the microorganisms. For instance, plasmids could carry genes that code for drug resistance, virulence, production of antimicrobial agents and metabolic activities (Ibrahim *et al.,* 2012). Advances in control of infection have not eliminated the risk of post-operative wound infections due to emergence and spread of resistant microbes. The condition is serious particularly in developing countries where there is irrational prescription of antimicrobials and no proper infection control program on ground. Therefore, there is a growing need for strategies to enhance rational use of antibiotics, new antimicrobial production and better infection control program which have been suggested to reduce the problem (de With *et al.,* 2016).

Again, limited information about the epidemiology of bacteria pathogens causing post-surgical wound infection and their drug susceptibility profile in Nigeria make rational use of drugs impracticable. Also, most of the organisms isolated from wounds have been found to be resistant to some commonly used antibiotics probably as a result of indiscriminate use of such drugs by patients. Therefore, this research was aimed at establishing likely bacterial isolates from post and non operative wound sepsis in patients attending Federal Medical Centre Keffi and to ascertain the susceptibility or resistance of such isolates to conventional antimicrobials and some newly developed ones.

METHODOLOGY

One hundred pus specimens were collected at Federal Medical Hospital Keffi, from operative and non-operative wound sepsis aseptically from patients in the surgical wounds in the different wards (in patient) and some out-patients by means of sterile swabs with globed hands gently but firmly avoiding a secondary injury to the collection site. It was carefully removed and immediately fixed back to the container. This collection was done before the early morning dressing at the designated wards. One of us is a staff working with the Federal Medical Centre, Keffi (our study area) and no application for ethnical clearance was made but the consent of the patients was obtained and the bench work was done according to National Committee for Clinical Laboratory Standards (NCCLS).

Samples processing

Transportation

All swab specimens were transported immediately to Microbiology Laboratory of Federal Medical Centre, Keffi and processed within two hours of collection.

Culturing

The specimens on reaching the laboratory were cultured on blood agar and MacConkey agar respectively. They were incubated aerobically for 24 h at 37°C. The following day the plates were examined macroscopically for colonial morphological characteristic. After which the colonies were grams stained and also subjected to motility test by hanging drop method.

Motility test

Motility test was performed by hanging drop method on rod-like organisms. The test organisms were suspended in peptone water and incubated at 37°C for three hours. A rising of plasticine with a diameter of about 2 cm was prepared on a glass slide a loopful of the culture was transferred to the centre of the cover slip and the ring of the plasticine made on the slide was gently pressed on the cover slip ensuring that the drop of the culture was in the centre of the ring and not in contact with it.

This was then inverted swiftly and examined under microscope with low power objective lens. A truly motile organism would be seen moving around through the medium and changing direction as compared to Brownian movement or convection current.

Biochemical reaction and identification

The organisms isolated were subjected to biochemical tests which included, sugar, fermentation tests, oxidase test, catalase, coagulase, urea hydrolysis and in dole production.

Catalase test

With the edge of a glass slide the colony was brought in contact with a drop of hydrogen peroxide on the slide. Effervescence occurs immediately which indicates positive reactions, while the absence of such reaction indicates negative reactions; $2H_2O_2$ (aq) → $2H_2O$ (l) + O_2 (g)

Coagulase test

The gram positive cocci that tested positive to catalase test were subjected to coagulase test. On clean grease free glass slide, a drop of physiological balimen was placed and homogenously with the test organism and after which a drop of plasma was dropped by the side of the slide and they are all mixed together properly. The presence of agglutination means that it is a positive reaction but the absence of it is a negative reaction.

Indole test

A discrete colony from a 24 h culture was inoculated in the peptone water and this was incubated for 24 h at 37°C. A few drops of Kovac's reagent was added and then shaken thoroughly. Formation of a pinkish ring which normally persist after shaking indicates a positive reaction, no pinkish ring formation indicates a negative reaction.

Oxidase test

A fair drops of the reagent, 1% solution of Tetramethyl-p-phenylenediamine dihydrochloride was applied to a small area of Whatman No 1 and a representative colony smeared on that moisture portion of the filter paper with the acid of the edge of clean glass slide the appearance of a deep purple colour is *indicative of positive reaction*.

Urease test

Urease slants were inoculated with the organism isolated and these were incubated aerobically at 37°C for 24 h. Urease slope remained yellow indicating a negative reaction, if Urease was produced, it would split urea to ammonia and carbon monoxide and change the colour of the indicator from yellow to pink.

Sugar fermentation test

The organism isolated was aseptically inoculated into the different peptone water sugars and they were incubated for 24 h of 37°C. They were read the next day for acid and gas production.

Antibiotic susceptibility (antibiogram)

The pure bacterial isolates were seeded on anti-sterient agar plates and the antibiotics disc at the appropriate antibiotic concentrations placed equidistantly from one another on the surface of the seeded plates. The plates were then incubated at 37°C for 24 h and the diameters of zones of inhibitions measured.

RESULTS AND DISCUSSION

Of the 100 swab samples aseptically collected from both operative and non-operative wound sepsis, in Federal Medical Centre Keffi, the occurrence of *Staphylococcus aureus* ranked highest (64.70%) amongst the bacterial isolates detected, followed by *Pseu domonas aeruginosa* (29.41%) and lastly *Escherichia coli* (5.88%) (Table 1). Males accounted for 70% of the samples while females accounted for 30% (Table 2). The *invitro* antibiotic susceptibility profile of *Staphylococcus aureus* in relation to diameter of zone of inhibition showed that cloxacillin ranked the best (72.7%) and the least were ampicillin and penicillin (4.5% each) while the organism was resistant to streptomycin and chloramphenicol (Table 3). For *Pseudomonas aeruginosa* susceptibility to ciprofloxacin had the highest *invitro* efficacy (75%) with streptomycin (5%) being the least while the organisms were resistant to penicillin and chloramphenicol (Table 4). Antibiogram on *Escherichia coli* revealed that norbactin was most efficacious (75%) while chloramphenicol recorded the least efficacy (50%) (Table 5). Wound sepsis in some rural parts of Nasarawa State is as a result of low level of personal hygiene coupled probably with poverty. *Staphylococcus aureus* ranking highest in occurrence compared with other bacterial isolates encountered could be attributed to the

Table 1. Occurrence of bacterial isolates from the samples.

Organism	Occurrence	Percentage
Staphylococcus aureus	44	58.67
Pseudomonas aeruginosa	20	26.67
Escherichia coli	11	14.67
Total	75	100

Table 2. Sex relationship of bacterial isolates.

	***Staphylococcus* aureus**	***Pseudomonas aeruginosa***	***Escherichia* coli**	**Total percentage**
Male	37	12	4	53 (77.94)
Female	7	8	0	15 (22.06)
Total	44	20	4	68 (100)

Table 3. *Invitr*o antibiotic susceptibility profile of *staphylococcus aureus* isolates.

Antibiotics (10 μg)	No. of Isolates	≤15mm	≥16mm	% Suscep	% Resistance
Ampicillin	44	44	0	0	100
Penicillin	44	44	0	0	100
Gentamycin	44	33	11	25	75
Tetracycline	44	38	6	13.64	86.36
Streptomicin	44	44	0	0	100
Norbactin	44	32	12	27.28	72.72
Cloxacillin	44	21	23	52.27	47.73
Ciprofloxacin	44	28	16	36.36	63.64
Chloramphenicol	44	44	0	0	100

Key: Break point ≤15mm(Resistance), ≥16mm(susceptible), Suscep (Susceptibility).

Table 4. *Invitro* antibiotic susceptibility profile of p*seudomonas aeruginosa* isolate.

Antibiotic(10μg)	No. of Isolates	≤15mm	≥16mm	% Suscep	% Resistance
Gentamicin	20	12	8	40	60
Streptomicin	20	20	0	0	100
Norbactin	20	10	10	50	50
Ciprofloxacin	20	7	13	65	35
Chloramphenicol	20	20	0	0	100

Key:Break point ≤15mm (Resistance), ≥16mm (susceptible), ≤, ≥(zone of inhibition),Suscep (Susceptibility).

Table 5. *Invitro* susceptibility profile of *Escherichia coli* isolates.

Antibiotics	No. of (10μg)	isolates	≤15mm	≤16mm	% Resistance	% Susceptible
Ampicillin		4	10	0	100	0
Tetrecylin		4	10	0	100	0
Penicellin		4	10	0	100	0
Streptomicin		4	10	0	100	0
Norbactin		4	10	0	100	0
Cloxacillin		4	7	3	70	30
Chloramphenicol		4	8	2	80	20

Key: break point ≤15mm (Resistance), ≥16mm (susceptible), ≤, ≥ (zone of inhibition).

fact that *S.aureus* is a commensal of the skin as opposed to *Pseudomonas aeruginosa* and *Escherichia coli*. Therefore the tendency of these organisms to get to injured sites on the skin cannot be over ruled.

These findings agree with that of Lopiso *et al*. (2014) who isolated some organisms from wound sepsis in Hawassa Teaching and Referral Hospital, though a few other organisms were isolated alongside.

More of males were affected than females and this could be as a result of the males being exposed to farm work, fishing and other hazardous activities than their counterparts. The occurrence of gram negative bacteria, especially nitrobacteria may also be due to the contamination of wound by enteric organisms during surgical procedure and or faecal contamination thereafter, as majority of the operations were under taken on abdomen. These groups of organisms are also becoming endemic in hospital environment as they easily transfer from object to object resisting common antiseptics (Odedina *et al*., 2007; Amrita *et al*., 2010). The high susceptibility of *S.aureus* to cloxacillin (72.8%) but resisting ampicillin and penicillin (4.5% each agree with the assertion of Cheesbrough, (2000) that *S.aureus* particularly hospital strains are resistant to penicillin and some of its related penicillin derivatives as a result of the production of plas*mid coded β*-lactamase. The *in vitro* antimicrobial sensitivity studies have shown that isolates react differently to various antibiotics as documented by Biadglegne *et al*. (2009); Mulu *et al*. (2006) and Gebre, (2007). Most isolates normally exhibit higher rate of resistance to antibiotics such as ampicillin and penicillin. The higher susceptibility of about 90% of gram negative isolates to ciprofloxacin in Gondar as reported by Mulu *et al.* (2006) declined to 72.8% in this present study. This may indicate a dropping efficacy of the drug with time. Most of the isolates in the study as elsewhere (Mulugeta and Bayeh, 2011) were also multiple drug resistant.

Multiple drug resistance *E. coli* has been reported to be widely distributed in hospitals and is increasingly being isolated from community which contradicts this present finding. Multiple Drug Resistance (MDR) bacteria are the principle cause of failure in the treatment of infectious diseases, resulting in increase in the term and magnitude of morbidity, higher rates of mortality and greater health cost burden (Howard *et al*., 2003; Coates *et al.,* 2002). However, the high occurrence (64.70%) of *S. aureus* calls for urgent need to find out new antimicrobial agents to overcome this menace (Peralta *et al.,* 2007; Coates *et al.,* 2002; Rahman *et al*., 2011). The need to practice aseptic procedures and rational use of antimicrobial agents to minimize infection rate and emergence of drug resistance is critical (Lopiso *et al.,* 2014).

Conclusion

In conclusion, the findings showed that bacteria associated with Post and Non Operative Wound Sepsis in this study area are *staphylococcus aureus, Pseudomonas aeruginosa* and *E.coli* being the least. The likely drug of choice against *Staphylococcus aureus, Pseudomonas aeruginosa* and *E.coli* is cloxacillin, ciprofloxacin and norbactin respectively.

Recommendations

It is recommended that proper hygiene and care should be taken while treating accident victims and surgical patients so that infections should not be transmitted mechanically from one patient to another through the instruments used, knowing also that an organism like *Pseudomonas aeruginosa* which is an opportunistic organism can thrive on instrument, wet surfaces and even in disinfectants. Also, the etiologic agent involved in a particular wound sepsis should be determined and the antibiogram run on the identified agent for effective chemotherapy on patients with wound sepsis should be encouraged.

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