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Antibiotic Resistance Profile of Gram Negative Bacteria Isolated from Surgical Wounds in Minna, Bida, Kontagora and Suleja Areas of Niger State

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Abstract Antibiotics resistance profiles of bacteria from surgical wounds were investigated in four (4) General Hospitals (Bida, Kontagora, Minna and Suleja) in Niger State. Five hundred (500) samples (i.e. Two hundred (200) samples in Minna, One hundred (100) samples each Suleja, Kontagora and Bida) of wound exudates from these general hospitals, were analysed. The results showed the presence of *Klebsiella Pneumoniae, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus vulgaris* in the samples from the wounds. From the five hundred (500) samples collected from all locations, forty two (42) samples had *Kl. pneumoniae*, sixty four (64) samples had *Ps. aeruginosa*, fifty two (52) samples had *P. vulgaris* and one hundred and nine (109) samples had *E. coli. E. coli* was the most frequently isolated bacteria from wounds in all the locations, while *Kl. pneumoniae* was the least isolated from wounds in all the locations. All the bacteria were tested for sensitivity against tarivid, pefloxacin, ciprofloxacin, augmentin, gentamycin, streptomycin, ceporex, nalidixic acid, septrin, ampicillin, ampiclox, zinacef, amoxacillin, rocephin, erythromycin. Most of all the isolates were sensitive to ciprofloxacin, pefloxacin and Tarivid while others were resistant to remaining antibiotics. *E. coli, Strept. pyogenes* and *S. aureus* showed highest resistance profile and *P. vulgaris, Kl. pneumoniae* and *Ps. aeruginosa* showed least resistance profile to most antibiotics used.

Keywords Antibiotic, Resistance, Sensitive, Surgical Wound

1. Introduction

Wound is defined as an injury to any of the tissues of the body, especially the one that is caused by physical means and with interruption of continuity (Giacometti *et al*., 2000). The most common underlying event for all wounds is trauma. Trauma may be accidental or intentionally induced. The intentionally induced trauma category includes hospital – acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devices. Although, none intentionally induced, hospital-acquired wounds can be the pressure sores caused by ischemia. They are also referred to as decubitus ulcers (bedsore), and when such wounds become infected, they are often colonizing bacterial species (Giacometti *et al*., 2000).

Wound can be infected by a variety of microorganisms ranging from bacteria to fungi and parasites (Bowler *et al*., 2001). The common gram positive organisms are the β –hemolytic *Streptococcus – Strept. pyogenes* and *S. aureus.* The gram negative aerobic rods are *Ps. aeruginosa*. The facultative anaerobes include *Enterobacter* species, *E. coli*, *Klebsiella* species and *Proteus* species. The fungi are *Candida* species and *Aspergillus* species (Gus Gunzalez *et al.*,2006; Mordi and Momoh, 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* (MRSA) and Vancomycin resistant *Enterobacter* (VRE), polymicrobic flora and by fungi.

The knowledge of the causative agents of wound infection has proved to be helpful in the selection of empiric antimicrobial therapy and on infection control measures in hospitals (Shittu *et al*., 2004) are also useful in formulating rational antibiotic policy. The aim of this research therefore is to determine the antibiotic resistance profile of gram negative bacteria isolated from surgical wounds in Minna, Bida, Kontagora and Suleja areas of Niger State, Nigeria.

2. Materials and Methods

2.1. Collection of Samples

Wounds samples were collected from five hundred (500)

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patients that undergo surgical operation in four (4) general hospitals in Minna, Bida, Kontagora and Suleja areas of Niger State. 200 samples were collected from general hospital in Minna while 100 samples were collected each from Bida, Kontagora and Suleja general hospitals. The wound types included boils, whitlow, abscesses, cervicitis, trauma wounds, burns, systemic ulcers, insect bites and swelling of no specific etiology. These samples were transferred to the Microbiology laboratory of Federal University of Technology, Minna, Niger State, Nigeria for further analysis.

2.2. Characterization and Identification of the Isolates

The collected samples were streaked on freshly prepared nutrient agar plates and incubated aerobically and anaerobically at 37°C for 24 hours. Bacterial colonies differing in size, shape and colour were selected from the different plates and further subcultured on nutrient agar by the streak plate technique and incubated at 37°C for 24 hours after which, were maintained in agar slants for further characterization and identification. The bacterial isolates were characterized based on colonial and cell morphology, growth on differential/selective media and biochemical tests which include Gram's reaction, indole tests, methyl red, voges-proskauer, citrate utilization, motility, endospore, utilization of carbohydrates such as glucose, sucrose, mannitol, lactose and fructose, oxidase, catalase, coagulase and starch hydrolysis test (Oyeleke and Manga, 2008). The bacterial isolates were identified by comparing their characteristics with those of known taxonomy using the schemes of Cowan and Steel (1993).

2.3. Susceptibility of Isolates to Various Antibiotics

Antibiotic sensitivity tests were carried out on all isolates using paper (New Man England) disc diffusion technique. A total of 10 antibiotics were tested and 0.2ml of 12h peptone water culture of test organism was used to inoculate each organism on a dry sterile nutrient agar plate. The resistant profiles of bacteria isolated from surgical wounds were determined by standard methods.

The antibiotic discs used were gram negative sensitive as follows: tarivid, pefloxacin, ciprofloxacin, augmentin, gentamycin, streptomycin, ceporex, septrin, ampicillin. Nutrient agar was the media used. Each of the isolates was spread over the entire surface of the nutrient agar using a sterile glass spreader and allowed to dry for about 15 to 30min. The antibiotic discs were placed on agar using sterile forceps. The plates with the antibiotic discs were then incubated at 37°C for 24 hours to observe the zones of growth inhibition produced by the antibiotics and recorded immediately.

3. Result

3.1. Microorganisms Isolated from Samples at Each Location

Table 1 shows the gram negative bacteria isolated from wound samples in various general hospitals examined. *E. coli* had the highest occurrence in all four locations (22%) followed by *P. aeruginosa* (12.8%) while *Kl. pneumonia* had the least occurrence (8.4%).

3.2. Antibiotic Resistance of Gram Negative Bacteria in Minna

Table 2 shows the antibiotics resistance of gram negative bacteria isolated from General hospital Minna. *E. coli* was most resistant while *Kl. pneuomoniae* was least resistant to the antibiotics examined while *E. coli* was most susceptible to all the antibiotics and *P. vulgaris* was most susceptible to all the antibiotics.

3.3. Antibiotic Resistance of Gram Negative Bacteria in Bida

Table 3 shows the antibiotics resistance of gram negative bacteria isolated from General hospital Bida. *E. coli* was most resistant while *Kl. pneumoniae* was least resistant to the antibiotics examined while *E. coli* was most susceptible to all the antibiotics and *P. vulgaris* was most susceptible to all the antibiotics.

3.4. Antibiotic Resistance of Gram Negative Bacteria in Kontangora

Table 4 shows the antibiotics resistance of gram negative bacteria isolated from General hospital Kontagora. *E. coli* was most resistant while *Kl. pneuomoniae* was least resistant to the antibiotics examined while *E. coli* was most susceptible to all the antibiotics and *Ps. aeruginosa* was most susceptible to all the antibiotics.

3.5. Antibiotic Resistance of Gram Negative Bacteria in Suleja

Table 5 shows the antibiotics resistance of gram negative bacteria isolated from General hospital Kontagora. *E. coli* was most resistant while *Ps. aeruginosa* was least resistant to the antibiotics examined while *Ps. aeruginosa* was most susceptible to all the antibiotics and *Kl. pneumoniae* was most susceptible to all the antibiotics.

4. Discussion

The gram negative organisms isolated in wound infection were *E. coli*, *P. vulgaris*, *Ps. aeruginosa* and *Kl. pneumoniae*. Of all the organisms isolated, *E. coli* had the highest frequency of occurrence (22%) followed by *Ps. aeruginosa* (12.8%). This finding was in agreement with Siguan *et al.* (1987) and Olayinka *et al.* (2004) which stated that the common organisms isolated from surgical wound were gram-negative microbes comprising *P. aeruginosa*, *E. coli* and *Enterobacter* spp.

E. coli showed the highest resistance to the antibiotics considered in all the locations. This was in agreement with the reports of Olayinka *et al*. (2004) which stated that *E. coli* was the commonest agent of bacteraemia and the one showing the most striking changes in resistance, especially to septrin and ampicillin. High incidence of *E. coli* in wound infection was reported by Wazait *et al*. (2003). The alarming trend is the speed with which aminoglycoside and penicillin resistance is accumulating in *E. coli* all over Africa especially in Nigeria (Hefferman and Woodhouse, 2007). Besides, *E. coli* easily acquire resistance factor from environment and they are easily resistant to penicillin derivatives drug like ampicillin and oxacillin (Wazait *et al*., 2003).

Kl. pneumoniae showed resistance to all the antibiotics except tarivid and pefloxacin, in Bida. *Ps. aeruginosa* was resistant to ceporex in all locations except in Suleja. *P. aeruginosa* also showed no resistant to tarivid in Bida. *E. coli*, *Ps. aeruginosa* and *Kl. pneumoniae* can acquire resistance plasmid in a mixed culture. This explains further why most of these organisms are resistant to antibiotics (Nester *et al*., 2004).

Proteus vulgaris is most frequently recovered from immunocompromised patients or those on long-term antibiotic regimen. This organism was resistant to all the antibiotics in all the locations. This finding was contrary to the reports of Mordi and Momoh (2009) which stated that *P. vulgaris* was sensitive to all antibiotics except chloramphenicol.

Klebsiella pneumoniae was the least commonly isolated organisms amongst the Gram negative facultative anaerobic bacilli. This is however contrary to the observations of Kehinde *et al.* (2004) who claimed that *Kl. pneumoniae* was most predominant in surgical wounds. But this finding was in line with the reports of Stock and Wiedemann (2001) which stated that *Kl. pneumoniae* was the least isolated in surgical wounds.

Table 1. Microorganisms isolated from samples at each location

Locations	No of samples	*E. coli*	*P. aeruginosa*	*Kl. pneumonia*	*P. vulgaris*
Minna	200	39 (37.5%)	28 (24.04)	17(16.35)	20(19.23)
Bida	100	29 (48.33%)	13(21.67)	8(13.33)	10(16.67)
Kontagora	100	21 (47.73)	9 (20.45)	7(15.91)	7(15.91)
Suleja	100	21 (36.84%)	14(24.56)	10(17.45)	12(21.05)
Total	500	110 (22%)	64 (12.8%)	42 (8.4%)	49 (9.8%)

NB: Values in parenthesis are % occurrence of isolate

Table 2. Antibiotics Resistance of Gram Negative Bacteria from Minna General Hospital

Antibiotics	*Escherichia coli*		*Ps. aeruginosa*		*Kl. pneumoniae*		*P. vulgaris*	
	R	Su	R	Su	R	Su	R	Su
OFX	11	28	10	18	2	15	5	15
PEF	12	27	13	15	3	14	8	12
CPX	12	27	14	14	4	13	8	12
AU	13	26	8	20	5	12	6	14
CN	13	26	11	17	6	11	4	16
S	19	20	14	14	6	11	7	13
CEP	16	23	13	15	5	12	10	10
NA	18	21	14	14	1	16	10	10
SXT	20	19	11	17	2	15	6	14
PN	20	19	13	15	4	13	5	15
TOTAL	154	236	121	159	38	132	69	131

Key: OFX = tarivid, PEF = pefloxacin, CPX = ciprofloxacin, AU = augmentin, CN = gentamycin, S = streptomycin, CEP = ceporex, NA= nalidixic acid, SXT= septrin, PN=ampicillin, R = resistant, Su = susceptible

Table 3. Antibiotics Resistance of Gram Negative Bacteria Bida General Hospital

Antibiotics	*E. coli*		*Ps. aeruginosa*		*Kl. pneumoniae*		*P. vulgaris*	
	R	Su	R	Su	R	Su	R	Su
OFX	10	19	0	13	0	8	5	5
PEF	14	15	2	11	0	8	7	3
CPX	7	22	3	10	3	5	5	5
AU	14	15	5	8	5	3	5	5
CN	13	16	6	7	5	3	7	3
S	19	10	10	3	5	3	8	2
CEP	14	15	10	3	6	2	6	4
NA	14	15	13	0	4	4	8	2
SXT	18	11	9	4	4	4	8	2
PN	18	11	10	3	4	4	7	3
TOTAL	141	149	68	62	36	44	66	34

Key: OFX = tarivid, PEF = pefloxacin, CPX = ciprofloxacin, AU = augmentin, CN = gentamycin, S = streptomycin, CEP = ceporex, NA= nalidixic acid, SXT= Septrin, PN =Ampicillin, R = resistant, Su = susceptible

Table 4. Antibiotics Resistance of Gram Negative Bacteria from Kontagora General Hospital

Antibiotics	*Escherichia coli*		*Ps. aeruginosa*		*Kl. pneumoniae*		*P.vulgaris*	
	R	Su	R	Su	R	Su	R	Su
OFX	6	15	4	5	3	4	2	8
PEF	8	13	3	6	3	4	3	7
CPX	7	14	3	6	2	5	7	3
AU	11	10	6	3	1	6	7	3
CN	9	12	5	4	2	5	6	4
S	13	8	4	5	2	5	5	5
CEP	13	8	5	4	2	5	4	6
NA	15	6	5	4	4	3	4	6
SXT	14	7	6	3	3	4	7	3
PN	15	6	7	2	3	4	4	6
TOTAL	111	99	48	42	25	45	49	51

Key: OFX = tarivid, PEF = pefloxacin, CPX = ciprofloxacin, AU = augmentin, CN = gentamycin, S = streptomycin, CEP = ceporex, NA= nalidixic acid, SXT= septrin, PN =ampicillin, R = resistant, Su = susceptible

Table 5. Antibiotics Resistance of Gram Negative Bacteria from General Suleja Hospital

Antibiotics	*E. coli*		*Ps. aeruginosa*		*Kl. pneumoniae*		*P.vulgaris*	
	R	Su	R	Su	R	Su	R	Su
OFX	11	10	1	13	3	7	5	7
PEF	10	11	2	12	3	7	5	7
CPX	13	8	3	11	3	7	7	5
AU	10	11	4	10	2	8	5	7
CN	9	12	3	11	2	8	3	9
S	12	9	1	13	1	9	4	8
CEP	12	9	0	14	1	9	2	10
NA	10	11	1	13	2	8	6	6
SXT	10	11	2	12	3	7	4	8
PN	14	7	4	10	6	4	3	9
TOTAL	111	99	21	119	26	74	44	76

Key: OFX = tarivid, PEF = pefloxacin, CPX = ciprofloxacin, AU = augmentin, CN = gentamycin, S = streptomycin, CEP = ceporex,NA= nalidixic acid, SXT= septrin, PN =Ampicillin, R = resistant, Su = susceptible

The *Kl. pneumoniae* was susceptible or show no resistance to all the antibiotics in all the locations (Bida, Minna, Kontagora and Suleja). This finding was contrary to Anderl *et al.* (2000) which stated that *K. pneumoniae* was resistant to ciprofloxacin and ampicillin.

The incidence of wound infection however varies in terms of surgery, hospital, surgical procedure and most importantly from one patient to another (Nichols, 2001). Also, hospital patients receiving broad-spectrum antibiotics as prophylaxis are frequently colonized by *P. aeruginosa* in the lower intestinal tract (Olayinka *et al*., 2004).

5. Conclusions

The findings of this study suggest that bacterial resistance in surgical wound infections is becoming serious menace in all the locations/study area.

E. coli is still the most frequently involved pathogen, showing high resistance rates of bacteria isolated from surgical wounds.

Tarivid, ciprofloxacin and Pefloxacin are the best therapeutic options to treat these infections because of the resistant caused by these organisms.

Infections of the surgical wound by these bacteria are one of the most common and important cause of morbidity and mortality. The delay in recovery and subsequent increased length of hospital stay also has economic consequences. It has been estimated that each patient with a surgical site infection will require an additional six to seven (6-7) days in the hospital, which results in the doubling of hospital costs.

Early treatment: when antibacterial therapy is indeed necessary, it should be promptly initiated; inadequate use of antibacterial (e.g., doses that are too low, therapy ended prematurely) is a major factor for the selection of resistant strains

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