Prevalence of Bacterial and Fungal Isolates Associated with Road Traffic Accident In-Patients in General Hospitals in Niger State

ABSTRACT

Aim: This study was carried out to determine the prevalence and antimicrobial susceptibility of bacteria and fungi isolated from wounds of in-patients with road traffic accidents at four General hospitals in Niger State

Place and Duration of Study: Department of Microbiology, Federal University of Technology, Minna, Nigeria., between October 2017 and May 2018.

Methodology: Wound swabs were taken twice per patient, first swab at contact and second swab taken seven days post wound dressing. The outcome of 1000 wound swabs taken from 409 (267 male and 142 female) in-patients with road traffic accident wound treated in the General hospitals Bida, Minna, Kontagora and Suleja areas of Niger State, Nigeria.

Results: 405 (40.5%) samples were positive for bacteria and fungi pathogens while 595 (59.5%) were negative. 262 (80.6%) swabs yielded single isolates while 46 (14.2%) yielded double organisms and 17 (5.2%) yielded three or more organisms. Of the 405 positive cultures, 347 (85.7%) were bacterial isolates while 58 (14.3%) were fungal isolates. *Staphylococcus aureus* predominates (37.7%), followed by *Pseudomonas aeruginosa* (15.6%), E. coli (11.4%), *Streptococcus pyogenes* (9.4%), *Bacillus subtillis* (7.2%), *Klebsiella pneumonia* (4.4%), *Aspergillus niger* (4.2%), *Candida albicans* (3.7%), *Aspergillus flavous* (2.7%), *Candida pseudotropicallis* (2.2%) and *Mucor pusillus (*1.5%).

Conclusion: Most bacterial isolates were sensitive to Levofloxacin, Ciprofloxacin, Streptomycin and Gentamycin, while others showed some degree of resistance to the remaining antibiotics. *Klebsiella pneumonia* showed the highest resistance to all the antibiotics used. All the fungi isolated tested were highly sensitive to the antifungal drugs used except Griseofulvin.

Keywords: Bacteria, Fungi, RTA wounds, Antibiotics, General Hospitals

INTRODUCTION

Road traffic accident results in 1.2 million deaths and injuries about four times this figure worldwide every year [1]. The causes of traffic crashes and fatalities are varied. Three major categories have traditionally been identified as human, vehicle and highway infrastructure. Amongst these, the road user attitude and human disability have been found to account for more than 85 per cent [2]. Road accidents result in trauma. About half of deaths due to trauma are within the age range 15–45 years, and males are twice commonly affected than females [3]. Trauma is the fifth leading cause of significant disability [4], the sixth leading cause of death worldwide. The commonest causes of death due to trauma are central nervous system injury, followed by substantial blood loss [4]. Road traffic accident wound infection are wound infections sustained after automobile accident. Wound infection is caused by microorganisms ranging from bacteria to fungi [5]. Main predisposing factor to infection include poor personal hygiene, equipment, the environment and patients risk factors.

Despite the use of recent technology in the management of wound in our hospitals, wound infection still remains the most common cause of hospital infection and are associated with increased morbidity and death [6-8]. In our local settings, the clinicians are faced with challenges on issues regarding wound infection due to road traffic accident injury [9]. There is the problem of poor or improper handling of road traffic accident victims with injury, the presence of nosocomial infection during admission in the hospital, the possibility of drug-resistant organisms with its resultant sequels such as prolongation of hospital stay, increasing cost of treatment and a possible loss of function as a complication [9].

Because of the high prevalence of wound colonization and / or infection in road accident victims with injuries, a critical issue is to be aware of the different antimicrobial resistance patterns, selective usage of antimicrobials only when absolutely necessary so as to avoid changing the normal flora of the skin, in order not to result in multidrug-resistant flora [10]. However, this necessitates periodical assessment of the causative agents of road accident wound infection and their sensitivity profiles which is going to be of great use in the comprehensive treatment of such wound in Niger state.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was carried out in three General Hospitals in Minna, Bida, Kontagora and Suleja Local Government Areas of Niger state. Minna is the capital of Niger state with two Local Government Areas, Bosso and Chanchaga. Niger state is part of North Central Geopolitical Zone of Nigeria and is located between latitudes 8^{o} $20^{/}$ N and 11^{o} $30^{/}$ N and longitude 3^{o} $30^{/}$ E and 7^{o} $20^{/}$ E. This study area is made up of people with different ethnic groups living together.

2.2. Study Population

Random sampling technique was used to collect samples from accident victims. A total of 500 wound swab samples were collected from in-patients between the ages of 15 to 70 years of age with fresh road accidents injury brought to accident and emergency unit at three general hospital considered in the study.

2.3 Ethical Approval

Wound swab samples were obtained with full informed consent of the accident victims was sought and for subjects less than 18 years had their consent sought from their relatives or guidance. Clearance to conduct this research was sought from the ethics and research committee of the hospital management board, Minna. Also, absolute confidentiality and privacy was respected.

2.4 Demographic Information

Socio-demographic data such as age, sex, location of accident and vehicle(s) involved in the accident using standard questionnaires and kept confidential during the research.

2.5 Sample collection

Five hundred fresh wound swabs were collected from 409 patients within the space of 6 months. Specimens were collected by rubbing the lesion with a sterile swab stick. The swab sticks were introduced into an ice pack and then transported to the microbiology laboratory of Federal University of Technology Minna Niger state for analysis.

2.6 Sample Processing

The swab sticks were inoculated onto Nutrient Broth and incubated at 37^{o}C for 24 hours. It was then sub cultured onto Nutrient Agar and MacConkey Agar by picking the organism from the Nutrient Broth culture with wire loop and streaking and were incubated for 24 hours at 37^{o}C. The colonies were then sub cultured onto Nutrient Agar and incubated at 37^{o}C for 48 hours to obtain pure isolates. The pure isolates were then characterized by Gram's staining and biochemical tests using Bergey's Manual of Determinative Bacteriology [11]. Suspected bacterial species were characterized and identified according to standard bacteriological methods as highlighted by Chessbrough, [12] and Barrow and Feltham [13].

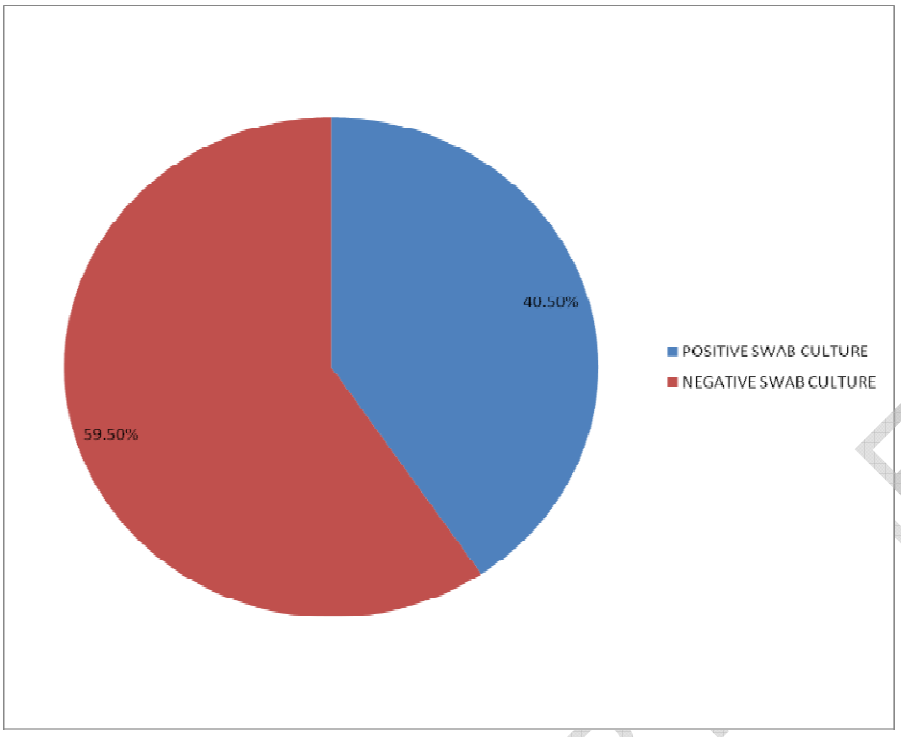
2.7 Antibiotic Susceptibility Test

Nutrient broth was prepared and inoculated with bacterial isolate (5ml of nutrient broth) using wire loop, and was incubated at 37^{o}C for 3 hours. After incubation, the turbid culture was compared with McFarland standard, and was smeared onto prepared nutrient agar using sterile cotton swab [12]. The commercial antibiotic susceptibility discs were picked with sterile forceps and placed on the surface of the inoculated nutrient agar under aseptic condition. The antibiotic discs used were Gram negative sensitivity as follows: tarivid, pefloxacin, ciprofloxacin, sparfloxacin, chloramphenicol, amoxicillin, Augmentin, gentamycin, streptomycin, septrin and Gram-positive sensitivity as follows: norfloxacin, streptomycin, levofloxacin, rifampicin, erythromycin, ampiclox, chloramphenicol, gentamycin, ciprofloxacin, amoxicillin. The plate containing the discs was incubated at 37^{o}C for 24 hours. The zones of inhibition were recorded accordingly and those that were resistant were also recorded. This was done for all the isolates.

3. RESULTS

Total of 1000 wound swabs from 409 patients with road accident wounds over the period of 6 months. All were in-patients in different wards in the hospital (ie Accident and Emergency ward, Emergency paediatric unit, intensive care unit (ICU), Surgical and Medical wards, Obstetrics and Gynaecology ward and Paediatrics ward).

The percentage of positive swab cultures as against negative swab cultures was shown in Figure 1. Of the 1000 swab samples taken from 409 patients, 267 were male and 142 were female, 405 (40.5%) samples showed growth for microorganisms while 595 (59.5%) showed no growth. Of the 405 microbial positive cultures, 347 (85.7%) were positive for bacteria, while 58 (14.3%) were positive for fungi as seen in Figure 2.



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122 **Figure 1:** Percentage of positive swab culture as against negative

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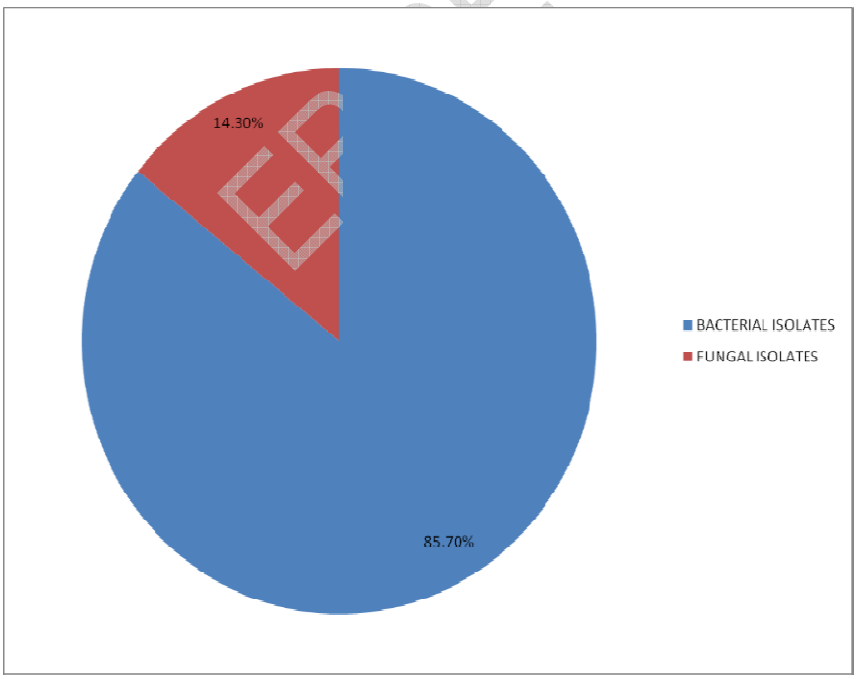

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125 **Figure 2**: Percentage of positive bacterial isolates in relation to positive fungal isolates

The number of organisms isolated per wound swab cultured, 262 (80.6%) of the microbial positive cultures showed single organism per wound swab, 46 (14.2%), showed double organisms per wound swab while 17 (5.2%) showed a mixture of three or more organisms per wound swab as shown in Figure 3.

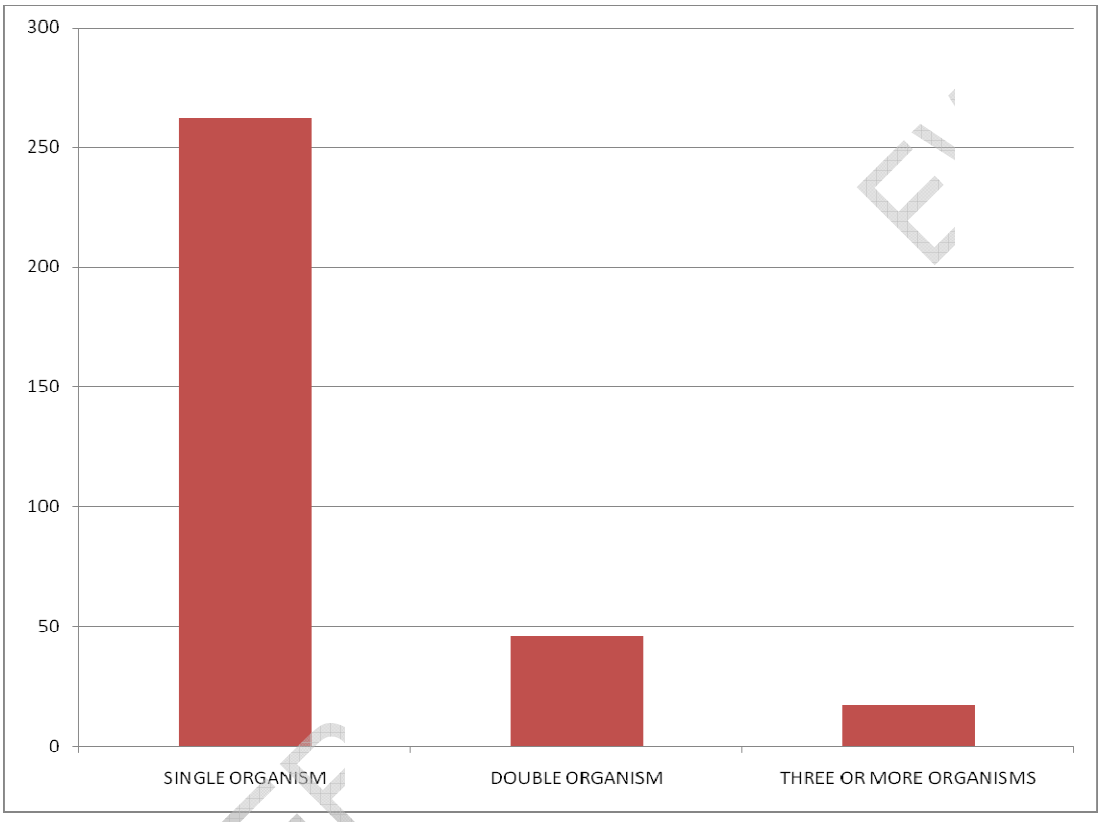

Figure 3: Distribution of Microorganism Isolated per Wound Swab Examined

Table 1 Shows the frequency of occurrence of the organisms isolated from road accident wounds over the period of 6 months. *Staphylococcus aureus* has the highest number of organisms with (37.7%), and is followed by *Pseudomonas aeruginosa* (15.6%), *E. coli* (11.4%), *Streptococcus pyogenes* (9.4%), *Bacillus subtilis* (7.2%), *Klebsiella pneumoniae* (4.4%), *Aspergillus niger* (4.2%), *Candida albicans* (3.7%), *Aspergillus flavus* (2.7%), *Candida pseudotropicalis* (2.2%) and *Mucor pusillus* (1.5%).

Table 1: Shows the Frequency of occurrence of Bacterial and Fungal Isolates

Isolates	Number of Isolates	Frequency of Occurrence (%)
Bacillus subtilis	29	7.2
Escherichia. coli	46	11.4
Klebsiella pneumoniae	18	4.4
Pseudomonas aeruginosa	63	15.6
Staphylococcus aureus	153	37.7
Streptococcus pyogenes	38	9.4
Aspergillus niger	17	4.2
Aspergillus flavus	11	2.7
Candida albicans	15	3.7
Candida pseudotropicalis	9	2.2
Mucor pusillus	6	1.5
Total	**4**05	100

Table 2 shows the Antibiotics sensitivity pattern exhibited by these microorganisms isolated from Road Accident Wound infections. Antibiotics sensitivity disk was used to carry out sensitivity test on each bacterial isolate. Gentamycin was sensitive to all bacteria isolated but resistant to Klebsiella spp. The zone of
inhibition was measured in diameter(mm) as: R-Resistance: 13 mm or less, I-Intermediate: 14-16 mm and S- Sensitive: 17 mm or more. The antibiotics used were: ST – Septrin, CH – Chloranphenicol, CX – Ciprofloxacin, SP – Sparfloxacin AX – Amoxacillin, AU – Augmentin, CN – Gentamycin, OF – Travid S – Streptomycin, PF – Pefloxacin, NB-Norfloxacin LF-Levofloxacin, RD-Rifampicin, E-Erythromycin, AX-Ampiclox (Table 2).

Furthermore, Table 3 shows the antifungal susceptibility pattern of the fungal isolates. Most of the antifungal drugs tested showed larger zones of inhibition, for instance itraconazole (40.5mm), ketoconazole (40.0mm), miconazole (39.0mm) and fluconazole (39.0mm). All the fungi isolated, showed no zones of inhibition that is resistant to the antifungal drug Griseofulvin

173 **Table 2:** Diameters of zones of inhibition (mm) of antibiotics susceptibility on bacterial

Bacteria	OF	PF	CX	SP	CH	AX	AU	CN	S	ST	NB	LF	RD	E	AX
Bacillus subtilis	*R*	*I*	*S*	*R*	*R*	*R*	*R*	*R*	*S*	*R*	*R*	*S*	*R*	*R*	*R*
Escherichia coli	*R*	*R*	*S*	*R*	*S*	*S*	*R*	*S*	*S*	*R*	*S*	*S*	*R*	*S*	*R*
Klebsiella pneumoniae	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*I*	*R*	*R*	*R*
Pseudomonas aeruginosa	*R*	*I*	*S*	*S*	*R*	*R*	*R*	*R*	*S*	*R*	*R*	*S*	*R*	*R*	*R*
Staphylococcus aureus	*I*	*S*	*R*	*R*	*I*	*I*	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*R*	*R*
Streptococcus pyogenes	*S*	*S*	*S*	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*R*	*S*	*R*

174 isolates from road accident wound infection

175 **R-Resistance: 13 mm or less, I-Intermediate: 14-16 mm and S- Sensitive: 17 mm or more.**

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178 **Table 3 Antifungal Susceptibility pattern on Fungal Isolates from Road Accident**

Fungi	Itraconazole (mm)	Ketoconazole (mm)	Miconazole (mm)	Fluconazole (mm)	Griseofulvin (mm)
A. niger	39.00	33.00	32.00	32.00	0.00
A. flavus	39.50	34.00	33.00	33.00	0.00
C. albicans	39.50	34.50	33.45	33.00	0.00
C. pseudotropicalis	37.50	32.60	31.50	31.00	0.00

M. Pusillus	40.50	40.00	39.00	39.00	0.00

4 DISCUSSION

This study was meant to explore the most common causes of wound infections associated with road traffics accident injury by examination of wound swabs. Of the 1000 wound swabs samples taken from 409 (267 males and 142 females) patients with road traffic accident wounds, 595 (59.5%) showed no microbial growth and 405 (40.5%) showed microbial growth. The age range that is mostly involved in road traffic accident according to this study is from 21 years to 40 years. This could be due to the fact that this age group constitute the most economic productive age bracket. This is similar to the findings of Chang [14], who attributed the Of the 409 cases of road traffic accidents recorded in this study, 280 cases were of motorcycle involvement in one way or the other and the most vulnerable route is Kotangora.

The most frequently isolated microorganisms were *Staphylococcus aureus* (37.7%), *Pseudomonas aeruginosa* (15.6%) and *Escherichia coli* (11.4%). This observation is in agreement with previous reports in Akwa Ibom and Cross River States, Nigeria [15, 16, 8]. General Hospital Minna showed the highest number of patients with swab specimen. This may be due to the fact that General Hospital Minna is in the capital and is with the largest number of bed space and serves as the referral centre to the other General Hospitals in the State. General Hospital Bida showed the least number of patients with swab specimens and this may be due to the fact that General Hospital Bida is in the same location with Federal Medical Centre Bida which serves as a referral centre for most accident cases in the area.

Staphylococcus aureus is the commonest isolated organism as it confirms the ubiquitous nature of *Staphylococcus aureus,* which is found in the human skin as a normal flora. *Bacillus subtilis* though not an organism commonly found in wound, but could be found in air, soil and water and hence can easily infect wound. *Candida albicans* is found in the vagina, but its presence in wound may be traced to immunocompromised state such as patients with diabetes mellitus, on prolonged steroid or prolonged use of antibiotics.

Klebsiella pneumoniae showed the highest resistance to all the antibiotics used, except for Levofloxacin. All the fungal organisms isolated in this study showed marked resistance against the antifungal drug Griseofulvin. Griseofulvin is known to act by binding to keratin tissue which is absent in wound. Other antibiotics and antifungals used in this study showed moderate to high sensitivity. Finally, the antibiotics: Levofloxacin, Ciprofloxacin, Streptomycin, Gentamycin and the antifungal: Itraconazole, Miconazole and Fluconazole are the most sensitive drugs as shown in this study, hence are recommended for use as empirical drug treatment pending the outcome of laboratory sensitivity result.

5. CONCLUSION

The findings of this study show that wound infections due to road traffic accidents is increasing and is becoming a problem in all the locations and areas studied. Many organisms were isolated during the period of study. Most of the bacteria were resistant to the antibiotics used, for instance the bacteria *Klebsiella pneumoniae* was resistant to all the antibiotics used except for levofloxacin.

Fungi isolated in this study were resistant to the fungicide Griseofulvin, but highly sensitive to the antifungal drugs Itraconazole, ketoconazole, Miconazole and Fluconazole. Levofloxacin, Ciprofloxacin, Streptomycin and Gentamycin are the most effective antibiotics used in this study and hence are the best drugs of choice for the empirical treatment of injuries and resistance due to road traffic accidents wound infection.

COMPETING INTERESTS

Authors have declared that there are no competing interests exist regarding this work.

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