1

5

9 10 ABSTRACT

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

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 pneumoniae* showed the highest resistance to all the antibiotics used. All the fungi isolated tested were highly sensitive to the antifungal drugs used except Griseofulvin.

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

13 14 INTRODUCTION

15

11

16 Road traffic accident results in 1.2 million deaths and injuries about four times this figure 17 worldwide every year [1]. The causes of traffic crashes and fatalities are varied. Three major 18 categories have traditionally been identified as human, vehicle and highway infrastructure. 19 Amongst these, the road user attitude and human disability have been found to account for 20 more than 85 per cent [2]. Road accidents result in trauma. About half of deaths due to 21 trauma are within the age range 15-45 years, and males are twice commonly affected than 22 females [3]. Trauma is the fifth leading cause of significant disability [4], the sixth leading 23 cause of death worldwide. The commonest causes of death due to trauma are central 24 nervous system injury, followed by substantial blood loss [4]. Road traffic accident wound 25 infection are wound infections sustained after automobile accident. Wound infection is caused by microorganisms ranging from bacteria to fungi [5]. Main predisposing factor to 26 27 infection include poor personal hygiene, equipment, the environment and patients risk 28 factors.

Despite the use of recent technology in the management of wound in our hospitals, wound 29 30 infection still remains the most common cause of hospital infection and are associated with 31 increased morbidity and death [6-8]. In our local settings, the clinicians are faced with 32 challenges on issues regarding wound infection due to road traffic accident injury [9]. There 33 is the problem of poor or improper handling of road traffic accident victims with injury, the 34 presence of nosocomial infection during admission in the hospital, the possibility of drug-35 resistant organisms with its resultant sequels such as prolongation of hospital stay, 36 increasing cost of treatment and a possible loss of function as a complication [9].

37 Because of the high prevalence of wound colonization and / or infection in road accident 38 victims with injuries, a critical issue is to be aware of the different antimicrobial resistance 39 patterns, selective usage of antimicrobials only when absolutely necessary so as to avoid 40 changing the normal flora of the skin, in order not to result in multidrug-resistant flora [10]. 41 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 42 43 comprehensive treatment of such wound in Niger state. 44

45 2.0 MATERIALS AND METHODS 46

47 2.1 Study Area

48 The study was carried out in three General Hospitals in Minna, Bida, Kontagora and Suleja 49 Local Government Areas of Niger state. Minna is the capital of Niger state with two Local 50 51 Government Areas, Bosso and Chanchaga. Niger state is part of North Central Geopolitical 52 Zone of Nigeria and is located between latitudes 8° 20' N and 11° 30' N and longitude 3° 30' 53 E and 7° 20' E. This study area is made up of people with different ethnic groups living 54 together. 55

2.2. Study Population

Random sampling technique was used to collect samples from accident victims. A total of 58 59 500 wound swab samples were collected from in-patients between the ages of 15 to 70 60 years of age with fresh road accidents injury brought to accident and emergency unit at three general hospital considered in the study. 61 62

63 2.3 Ethical Approval

65 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 66 67 guidance. Clearance to conduct this research was sought from the ethics and research 68 committee of the hospital management board, Minna. Also, absolute confidentiality and 69 privacy was respected. 70

2.4 Demographic Information

73 Socio-demographic data such as age, sex, location of accident and vehicle(s) involved in the 74 accident using standard questionnaires and kept confidential during the research. 75

76 2.5 Sample collection

77

56

57

64

71

72

78 Five hundred fresh wound swabs were collected from 409 patients within the space of 6

79 months. Specimens were collected by rubbing the lesion with a sterile swab stick. The swab 80 sticks were introduced into an ice pack and then transported to the microbiology laboratory 81 of Federal University of Technology Minna Niger state for analysis.

82 2.6 Sample Processing

83 84 The swab sticks were inoculated onto Nutrient Broth and incubated at 37°C for 24 hours. It 85 was then sub cultured onto Nutrient Agar and MacConkey Agar by picking the organism 86 from the Nutrient Broth culture with wire loop and streaking and were incubated for 24 hours at 37°C. The colonies were then sub cultured onto Nutrient Agar and incubated at 37°C for 87 88 48 hours to obtain pure isolates. The pure isolates were then characterized by Gram's 89 staining and biochemical tests using Bergey's Manual of Determinative Bacteriology [11]. 90 Suspected bacterial species were characterized and identified according to standard 91 bacteriological methods as highlighted by Chessbrough, [12] and Barrow and Feltham [13]. 92

93 2.7 Antibiotic Susceptibility Test

94 95 Nutrient broth was prepared and inoculated with bacterial isolate (5ml of nutrient broth) using wire loop, and was incubated at 37°C for 3 hours. After incubation, the turbid culture was 96 97 compared with McFarland standard, and was smeared onto prepared nutrient agar using 98 sterile cotton swab [12]. The commercial antibiotic susceptibility discs were picked with 99 sterile forceps and placed on the surface of the inoculated nutrient agar under aseptic 100 condition. The antibiotic discs used were Gram negative sensitivity as follows: tarivid, 101 pefloxacin, ciprofloxacin, sparfloxacin, chloramphenicol, amoxicillin, Augmentin, gentamycin, 102 streptomycin, septrin and Gram-positive sensitivity as follows: norfloxacin, streptomycin, levofloxacin, rifampicin, erythromycin, ampiclox, chloramphenicol, gentamycin, ciprofloxacin, 103 104 amoxicillin. The plate containing the discs was incubated at 37°C for 24 hours. The zones of 105 inhibition were recorded accordingly and those that were resistant were also recorded. This 106 was done for all the isolates. 107

108 3. RESULTS

109

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.

120









134 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%).

151	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	405	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	СХ	SP	СН	АХ	AU	CN	S	ST	NB	LF	RD	E AX
Bacillus subtilis	R	Ι	S	R	R	R	R	R	S	R	R	S	R	RR
Escherichia coli	R	R	S	R	S	S	R	S	S	R	S	S	R	S R
Klebsiella	R	R	R	R	R	R	R	R	R	R	R	I	R	RR
pneumoniae														
Pseudomonas	R	Ι	S	S	R	R	R	R	S	R	R	S	R	R R
aeruginosa									4					
Staphylococcus	Ι	S	R	R	Ι	Ι	R	1	S	R	1	S	R	RR
aureus							4	\bigcirc		Vara P				
Streptococcus	S	S	S	R	R	R	R	S	S	S	S	S	R	SR
pyogenes					4		K							

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.

176 177 178

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

М. І	Pusil	llus
------	-------	------

39.00

180 4 DISCUSSION

This study was meant to explore the most common causes of wound infections associated 181 182 with road traffics accident injury by examination of wound swabs. Of the 1000 wound swabs 183 samples taken from 409 (267 males and 142 females) patients with road traffic accident 184 wounds, 595 (59.5%) showed no microbial growth and 405 (40.5%) showed microbial 185 growth. The age range that is mostly involved in road traffic accident according to this study 186 is from 21 years to 40 years. This could be due to the fact that this age group constitute the 187 most economic productive age bracket. This is similar to the findings of Chang [14], who 188 attributed the Of the 409 cases of road traffic accidents recorded in this study, 280 cases 189 were of motorcycle involvement in one way or the other and the most vulnerable route is 190 Kotangora.

40.00

191

179

192 The most frequently isolated microorganisms were Staphylococcus aureus (37.7%), 193 Pseudomonas aeruginosa (15.6%) and Escherichia coli (11.4%). This observation is in 194 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 195 196 may be due to the fact that General Hospital Minna is in the capital and is with the largest 197 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 198 199 this may be due to the fact that General Hospital Bida is in the same location with Federal 200 Medical Centre Bida which serves as a referral centre for most accident cases in the area. 201

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.

209 Klebsiella pneumoniae showed the highest resistance to all the antibiotics used, except for 210 Levofloxacin. All the fungal organisms isolated in this study showed marked resistance 211 against the antifungal drug Griseofulvin. Griseofulvin is known to act by binding to keratin 212 tissue which is absent in wound. Other antibiotics and antifungals used in this study showed moderate to high sensitivity. Finally, the antibiotics: Levofloxacin, Ciprofloxacin, 213 214 Streptomycin, Gentamycin and the antifungal: Itraconazole, Miconazole and Fluconazole are 215 the most sensitive drugs as shown in this study, hence are recommended for use as 216 empirical drug treatment pending the outcome of laboratory sensitivity result.

218 5. CONCLUSION

219

217

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 <u>levofloxacinLevofloxacin</u>.

225

Formatted: Highlight

Fungi isolated in this study were resistant to the fungicide Griseofulvin, but highly sensitive to
 the antifungal drugs Itraconazole, <u>ketoconazoleKetoconazole</u>, 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.

REFERENCES

232 233

234 235 236

241 242

- World Health Organisation. World Report on Road Traffic Injury Prevention:
 Summary. World Health Organization, Geneva, Switzerland. 2004
- Odero W, Khayes M and Heda PM. Road Traffic Injuries in Kenya: Magnitude, Causes and Status of Intervention. Injury Control Safety Promotion. 2003; 10:53-61.
- 247 3. Porter SJ. and Greavis IK. Major trauma. UK: Oxford University Press. 2010.
- Palmer C. "Major trauma and the injury severity score—where should we set the bar?". Annual Proceedings of the Association for the Advancement of Automotive Medicine. 2007; 51:13–29. PMC 3217501. PMID 18184482.
- Bowler PG, Duerden, BI and Amstrong DC. Wound microbiology and associated approaches to wound management. Clin Microb Rev. 2001; 14:244 – 269
- Dionigi R, Rovera F, Dionigi G, Imperator A, Ferrari A and Dionigi R. Risk factors in surgery. J Chemoth. 2001; 13:6-11
- Iroha IR, Amadi ES and Orji JO. Esimone C O. In vitro evaluation of the activity of colloidal silver concentrate against *Pseudomonas aeruginosa* isolated from postoperative wound infection. Sci Res Essay. 2008; 3(5): 209-211
- Akinjogunla OJ, Adegoke A A, Mboto CI, Chukwudebelu IC and Udokang IP.
 Bacteriology of automobile accident wounds infection. Int J Med Med Sci. 2009; 1(2): 23-27
- Federal Road Safety Commission: Nigerian traffic accidents (January to October, 2009). FRSC Report 2009. Abuja: Federal Road Safety Commission; 2009.
- 263 10. Shittu AO, Kolawole DO and Oyedepo ER. A study of wound infections in two health
 264 institutions in Ile-Ife, Nigeria. Afr J Bio Res. 2002; 5:97-110.
- 11. Buchanan KE, Gibons NE. Bergey's manual of determinative bacteriology. (8th ed.)
 Williams and Williams company, Baltimoe. 1974.

- 267 12. Cheesbrough, M. Microscopical Techniques used in Microbiology. In District
 268 Laboratory Practice in Tropical Countries (Part 2). Third Edition United Kingdom:
 269 Cambridge University Press, 2010.
- Barrow GI and Feltham RKA. Bacterial characters and Characterization. In: Cowan and Steel's manual for identification of medical bacteria (3rd ed.) Cambridge University Press. The Edinburgh Building, Cambridge CB2 2RU, UK. 2003.
- 273 14. Chang D. Comparison of Crash Fatalities by Sex and Age Group. Traffic Safety
 274 Facts, National Center for Statistics and Analysis, 1200 New Jersey Avenue SE.,
 275 Washington, DC. 2008; 1-6.
- Anbumani N, Kalyan J and Mallike M. Epidemiology and Microbiology of wound infections. Ind J Prac Doc. 2006; 3(5):55 – 73.
- 16. Isibor JO, Oseni A, Eyaufe A, Osagie R and Turay A. Incidence of aerobic bacteria
 and Candida albicans in post-operative wound infections. Afr J Micr Res. 2008;
 2:288-291

MARRE