Antibiotic Susceptibility Profile of Microbial Isolates From Open Wounds To Common Antibiotics

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Abstract: Wound healing is a complex and dynamic process with the wound environment changing with the changing health status of the individual. This is a resultant effect of antibiotic resistance which the World Health Organization (WHO) has declared as pandemic since 2012. To consolidate this fact, forty-one (41) open wound swab samples were collected from Lagos State University Teaching Hospital (LASUTH)and analyzed microbiologically to identify to species level. The use of the microbact 12a/12b and 24 eidentification kits was employed. Fifty-four (54) bacterial isolates were isolated comprising of ten different bacteria organisms with their percentage prevalence, videlicet; *Staphylococcus aureus* (33.33%), *Pseudomonas aeruginosa* (20.37%), *Klebsiella pneumoniae* (14.81%), *Escherichia coli(9.25%), Acinetobacta iwojfi* (7.41%), *Klebsiella oxytoca* (5.56%), *Proteus mirabilis(3.10%), Proteus vulgaris* (1.85%), *Acinetobacta baumani* (1.85%) and *Escherichia coli-inactive* (1.85%). The isolates were cultured on MacConkey, mannitol salt, Eosin methylene blue and nutrient agar. Gram staining technique was used to determine the Gram- positive and Gram- negative bacteria, after culturing, sensitivity test was done on all the isolated bacteria with Mueller-Hilton agar using Kirby-bauer technique, and the following antibiotics ceftazidime (CAZ) 30ug, cefuroxime (CRX) 30ug, gentamicin (GEN) lOug, cefriaxone (CTR) 30ug, erytheomycin (ERY) 5ug, cloxacilin (CXC) 5ug, ofloxacin (OFL) 5ug, augmentin (AUG) 30u.g. Only ofloxaxin was found to be very effective followed by gentamicin, the rest of the antibiotics were ineffective against the microorganisms as they were multiple drug resistant.

Keywords: antibiotics, drug resistant, microbes, open wounds, sensitivity test

Introduction

A wound is defined as a physical injury where the skin or mucous membrane is torn, pierced, cut, or otherwise broken. The process of wound healing is complex and involves inflammatory, vascular, connective tissue and epithelial cells working together over a period of time (Kemebradikumo *et al*., 2013). To better understand wound healing one need to learn more about the different types of wounds and factors involved in their healing (**Velayati *et al*., 2009**). A wound may be described in many ways; by its etiology, anatomical location, by whether it is acute or chronic by the method of closure, by its presenting symptoms or indeed by the appearance of the predominant tissue types in the wound bed(Enoch and Price, 2004; Egbe *et al*., 2011). All definitions serve a critical purpose in the assessment and appropriate management of the wound through to symptom resolution or, if viable, healing. A wound by true definition is a breakdown in the protective function of the skin: the loss of continuity of epithelium, with or without loss of underlying connective tissue (that is muscle, bone, nerves)(Leaper and Harding, 1998) following injury to the skin or underlying tissues/ organs caused by surgery, a blow, a cut, chemicals, heat/ cold, friction/ shear force, pressure or as a result of disease, such as leg ulcers or carcinomas (Hutchinson, 1992).

Wound infection is one of the health problems that is caused and aggravated by the invasion of pathogenic organisms.

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Information on local pathogens and sensitivity to antimicrobial agents, and topical agents like acetic acid is crucial for successful treatment of wounds. Wound infections have been a problem in the field of surgery for a long time. Advances in control of infections have not completely eradicated this problem because of development of drug resistance. Antimicrobial resistance can increase complications and costs associated with procedures and treatment (Anguzu *et al*., 2007).Since the 1940s, these drugs have greatly reduced illness and death from infectious diseases. However, these drugs have been used so widely and for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making the drugs less effective,

Antibiotic / Antimicrobial resistance is the ability of microbes to resist the effects of drugs – that is, the germs are not killed, and their growth is not stopped. Although some people are at greater risk than others, no one can completely avoid the risk of antibiotic-resistant infections. Infections with resistant organisms are difficult to treat; requiring costly and sometimes toxic alternatives. Bacteria will inevitably find ways of resisting the antibiotics developed by humans, which is why aggressive action is needed now to keep new resistance from developing and to prevent the resistance that already exists from spreading (CDC, 2017). Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance occurs when bacteria change in response to the use of these medicines. Bacteria, not humans or animals, become antibiotic-resistant. These bacteria may infect humans and animals, and the infections they cause are harder to

Table 3: Biochemical Test and Identification of Isolates using Microbact for Non-Lactose Fermenters, 12B/24E

LYS	ORN	H_2S	GLU	MNT	XYL	ONP	IND	URE	V-P	CTR	TDA	GLT	MLT	ISL	SBT	RMS	SUC	LAC	ABN	ADT	RFS	SLC	AGN	ORGANISMS
-	-	+	-	-	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	*Acinetobacta iwoffi*
+	-	+	+	+	-	+	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+	*Pseudomonas aeruginosa*
+	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Pseudomonas aeruginosa*
-	-	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	*Acinetobacta iwoffii*
+	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Pseudomonas aeruginosa*
-	-	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	*Acinetobacta iwoffii*
+		-	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Pseudomonas aeruginosa*
+	-	-	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	*Pseudomonas aeruginosa*
+	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Pseudomonas aeruginosa*
-	-	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	*Acinetobacta iwoffii*
+	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Pseudomonas aeruginosa*
+	-	+	+	+	-	+	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+	*Pseudomonas aeruginosa*
-	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	*Actinetobactabaumani*

Table 4: Confirmation of all the suspected *Staphylococcus aureus* with Biochemical Tests

catalase	coagulase	organism
+	+	*Staphylococcus aureus*

Table 5: Performance Standards for Antimicrobial Susceptibility Testing

Antimicrobial Agent	Disc content	Susceptible	Intermediate	Resistant
Ceftazidime	30 µg	≥18	15–17	≤14
Cefuroxime	30 µg	≥20	17–19	≤16
Gentamicin	10 µg	≥15	13–14	≤12
Ceftriaxone	30 µg	≥21	14–20	≤13
Erythromycin	15 µg	≥23	14–22	≤13
Cloxacillin	5µg	≥17	14–16	≤13
Ofloxacin	5µg	≥16	13–15	≤12
Augmentin	30 µg	≥19	16–18	≤15

Table 6: Antibiotic Resistance Profile of the Bacterial Isolates.

Organisms	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Ss1*(P.aeriginosa)*	R	R	S	R	R	R	R	R
Ss2 (*S.aureus*)	R	R	S	R	R	S	R	R
Ss3 (*K.oxytoca*)	R	S	R	R	R	R	S	R
Ss4 (*S.aureus*)	R	R	S	R	R	R	S	R
Ss5*(E.coli)*	R	R	S	R	S	R	S	R
Ss6*(K.pneumoniae)*	R	R	S	R	R	R	S	R
Ss7 (*A.baumanni*)	R	R	S	R	R	R	R	R
Ss8 (*S.aureus*)	R	R	S	R	R	R	S	R
Ss9 (*E.coli-inactive*)	R	R	S	R	S	S	S	S
Ss10 (*P.vulgaris*)	R	R	S	S	R	R	S	R
Ss11 (*S.aureus*)	R	R	R	R	R	R	R	R
Ss12(*K.oxytoca*)	R	R	S	R	R	R	S	R
Ss13(*S.aureus*)	R	R	S	R	R	S	S	R
Ss14(*E.coli*)	R	R	S	R	R	R	S	R
Ss15(*P.aeriginosa*)	R	R	R	R	R	R	R	R
Ss16(*P.aeriginosa*)	R	R	S	R	S	S	S	R
Ss17(*E.coli*)	R	R	S	R	S	R	S	S
Ss18(*K.pneumoniae*	R	R	S	R	R	R	S	S
Ss19(*P.mirabilis*)	R	R	S	S	R	S	S	S

Key: R=resistance, S=susceptible

Table 7: Antibiotic Resistance Profile of the Isolates.

Organisms	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Ss20 (*K.oxytoka*)	R	R	S	R	R	S	S	S
Ss21 (*A.iwoffi*)	R	R	S	R	R	R	S	S
Ss22 (*P.aeruginosa*)	R	R	S	R	R	R	S	R
Ss23 (*S.aureus*)	R	R	S	R	R	R	S	R
Ss24 (*S.aureus*)	R	R	S	R	S	R	S	R
Ss25 (*E.coli*)	R	R	S	R	R	R	S	R
Ss26(*K.pneumoniae*)	R	S	S	R	R	R	S	R
Ss27 (*S.aeruginosa*)	S	S	R	S	S	R	S	S
Ss28 (*S.aureus*)	R	R	S	R	R	R	S	R
Ss29 (*S.aureus*)	R	R	S	R	R	R	S	R
Ss30(*K.pneumoniae*)	R	R	S	R	R	R	S	R
Ss31 (*S.aureus*)	R	S	S	S	S	R	S	S
Ss32 (*S.aureus*)	R	R	S	R	S	R	S	S
Ss33 (*S.aureus*)	R	R	R	R	R	R	S	R
Ss34 (*P.aeriginosa*)	R	R	S	R	S	S	S	S
Ss35(*K.pneumoniae*)	S	S	R	S	R	S	S	S
Ss36 (*P.aeriginosa*)	R	R	S	R	R	R	S	R
Ss37 (*A.iwoffii*)	R	S	R	R	R	S	S	S
Ss38(*K.pneumoniae*)	R	S	R	S	R	S	S	S
Ss39 (*P.aeriginosa*)	R	R	R	R	R	R	S	R
Ss40 (*S.aureus*)	R	S	S	R	S	R	S	R
Ss41 (*S.aureus*)	R	R	S	R	S	R	S	R
Ss42 (*A.iwoffii*)	R	R	S	R	S	S	S	R
Ss43 (*S.aureus*)	S	R	R	S	S	R	S	S
Ss44 (*P.aeriginosa*)	R	R	S	S	S	R	S	S

Ss45(*K.pneumoniae*)	S	S	R	S	S	R	S	S
Ss46 (*S.aureus*)	R	S	R	R	S	S	S	S
Ss47 (*S.aureus*)	R	R	S	S	S	S	R	S
Ss48 (*P.aeriginosa*)	R	R	R	R	R	R	S	R
Ss49 (*S.aureus*)	R	S	R	S	R	R	S	R
Ss50(*K.pneumoniae*)	R	R	R	R	R	R	S	R
Ss51 (*E.coli*)	R	S	S	R	S	S	S	S
Ss52 (*P.aeriginosa*)	R	S	R	S	S	R	S	S
Ss53 (*A.iwoffii*)	R	S	S	S	S	R	S	S
Ss54(*K.pneumoniae*)	S	S	S	S	S	R	S	S

Key: CAZ =Ceftazidime, CRX=Cefuroxime, GEN=Gentamicin, CTR=Cefriaxone, ERY=Erythromycin, CXC=Cloxacilin, OFL=Ofloxacin, AUG=Augmentin

Table 8: Five Different Classes of the Eight Antibiotics Used for the Research.

Antibiotics used	*Abbreviation*	*Class of antibiotic*
Ceftazidime 30µg	CAZ	Cephalosporins
Ceftriaxone 30µg	CTR	Cephalosporins
Cefuroxime 30µg	CXM	Cephalosporins
Erythromicin 15µg	ERY	Macrolide
Gentamicin 10µg	GEN	Aminoglycoside
Ofloxacin 5µg	OFL	Quinolones and Fluoroquinolones
Cloxacilin 5µg	CXC	Penicilin
Augmentin 30µg	AUG	Penicilin

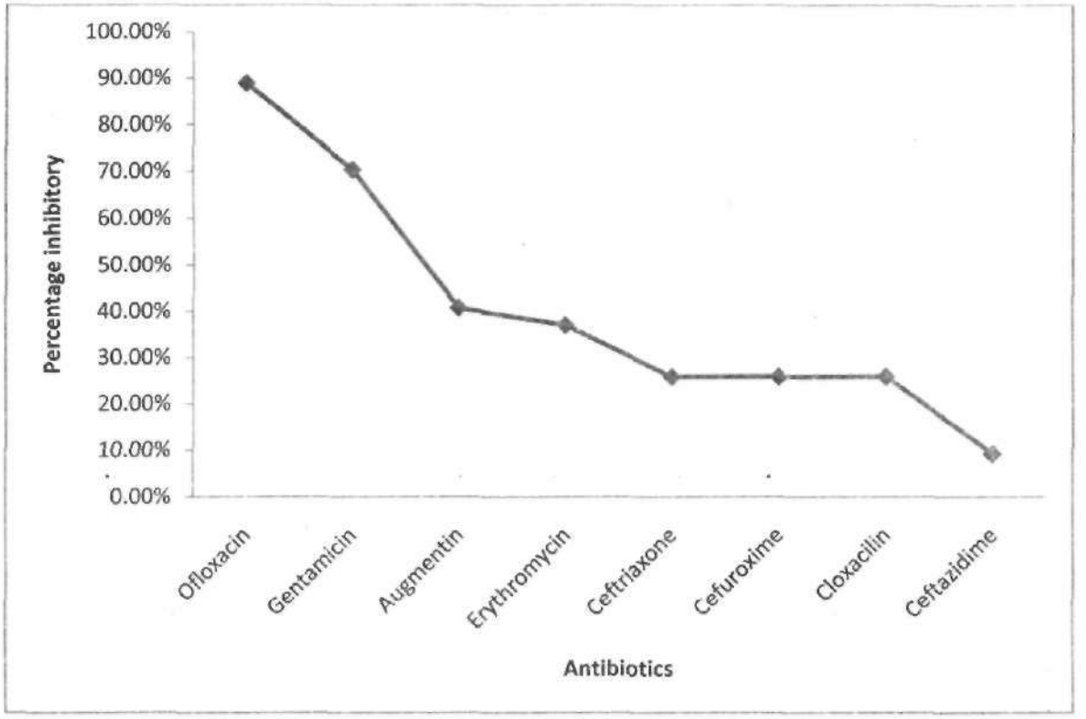

Figure 1: The percentage inhibitory activities of the antibiotics used

Discussion

Man has used different plants against common illness prevailing in the society with varying degree of success. The knowledge of drugs has developed along with the evolution of scientific and social progress. Drugs which are extracted from plants are very effective, easily available and less expensive and they rarely have side effects to the users. The up growing resistance of microorganisms to the conventional antimicrobial agents is a source of great concern to clinical microbiologists (Bhat and Vasaikar, 2010; Velayati*et al*., 2009). Bacteria evolve some changes in their genome with time, as a result, a large number of bacteria species particularly *Shigella* and *Escherichia coli* have become resistant to the antibacterial drugs due to extensive use and often create a problem in treatment of infectious disease (Walsh, 2003).Thus, it is the need of the hour to find out the antibiotics that are therefore, ineffective and the organisms that are resistant against them. Bacterial contamination of wounds is a serious problem in the hospital, especially in surgical practice where the site of a sterile operation can become contaminated and subsequently infected (Odelowo and Onile, 1990). This study demonstrated a high prevalence of pathogenic bacteria in wounds. This finding is consistent with that obtained in similar studies in Nigeria (Wariso *et al*., 2003; Sule *et al*., 2012; Ohalete *et al*., 2012; Sani *et al*., 2012).

This study has revealed the presence of 54 bacterial isolates comprising of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Actinetobactaiwoffi*, *Klebsiellaoxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, *Actinetobactabuamanni* and *Escherichia coli-inactive* in the order of prevalence respectively; 33.33%, 20.37%, 14.81%, 9.25%, 7.41%, 5.56%, 3.70%, 1.85%, 1.85% and 1.85%. The bacterium with the highest prevalence is *Staphylococcus aureus* with 33.33%and the least prevalent are *Proteus vulgaris*, *Actinetobactabuamanni*, and *Escherichia coli-inactive* with 1.85%. These bacteria have been previously reported as the implicated microorganisms in wounds (Wariso *et al*., 2003). Of the ten different species of organism isolated, nine of them are gram negative, while *Staphylococcus aureus*is the only Gram positive organism isolated. This is similar to a previous study in which majority of the implicated microorganism in wound samples are reported to be gram negative (Jerry *et al*., 2018).This study has revealed that the most potent antibiotic against all bacteria as compared to other antibiotics used in for this research was ofloxacin, and the antibiotic with least activity was ceftazidime.

Conclusion

Resistant organisms had been reported as normal body flora in some individuals including health care workers (Nordmann *et al*., 2012). Individuals with resistant organism could be initiators of community acquired infections. This research has revealed that most bacteria implicated in the wounds are multiple drugs resistant, and that most common antibiotics are not effective against the bacteria again. It has also revealed that, the most prevalent bacterium implicated in wound is *Staphylococcus aureus* and the least

prevalent is *Proteus* spp. This research reveals that *Pseudomonas aeruginosa* is resistant to all the antibiotics used against it, and this finding is similar to the findings of another researcher that revealed that *Pseudomonas aeruginosa* exhibited a very high resistance to the tested antibiotics (Kemebradikumo *et al.*, 2013).

Recommendation

The world urgently needs to change the way it prescribes and uses antibiotics. Even if new medicines are developed, without behavioural change, antibiotic resistance will remain a major threat. Behavioural changes must also include actions to reduce the spread of infections through vaccination, hand washing, practising safer sex, and good food hygiene (WHO, 2016).

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