Plasmid Profile of Bacteria Isolated from tears of HIV/AIDS Patients

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SUMMARY

Objective: The purpose of this study is to determine the presence and transfer of plasmids in bacteria isolated from tears of HIV/AIDS patients, their sensitivity and resistance to commercially available antibiotics.

Design: This was a cross sectional experimental study.

Materials and methods: One hundred tears samples from HIV/AIDS patients and fifty tears samples from HIV/AIDS negative patients were screened for resistance to 14 commercially available antibiotics using disc diffusion method.

Result: Three multiple antibiotics resistant strains of *staphylococcus aureus* and four multiple antibiotics resistance strains of *Pseudomonas aeruginosa* were identified. *staphylococcus aureus* strains showed 100% resistance to Ampiclox and erythromycin, 66.6% to Perfloxacin, amoxicillin and septrin, 33.33% to ciprofloxacin. *Pseudomonas aeruginosa* strains showed 100% resistance to streptomycin, amoxicillin, septrin and chloramphenicol. Only I strain of *staphylococcus aureus* showed presence of plasmid which was not transferable to *Escherichia coli* because of presence of disulphide cross-linked cell wall. Other strains of both *staphylococcus aureus* and *Pseudomonas aeruginosa* remained resistant after curing .

Conclusion: Further studies are needed in this area to show if antibiotic resistance in HIV/AIDS positive patients could be as a result of plasmid as well as other factors.

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Key words: Plasmids, Resistance, antibiotics, bacteria.

INTRODUCTION

The remarkable success of antimicrobial drugs generated a misconception that infectious diseases had been conquered. However, infectious diseases remain the second leading cause of death worldwide. One of the ongoing problems that public health workers face in the fight against infections is the development of resistance to multiple antimicrobial drugs which has created a situation in which there are few or no treatment options for infections with certain microorganism.

Abuse and misuse of antibiotics have been found to play a major role in the development and spread of resistance, which is very common in HIV/AIDS patients because of the multiple drug regime used to manage the disease and its complications.[2] Bacteria that used to be treated with antibiotics are now problematic because of resistance.

Resistance to antibiotics can be spread by the transfer of resistant plasmids by bacteria carrying them to other bacteria lacking plasmids. Plasmids are small double stranded circular DNA molecules that exist independently of the host chromosomes and are capable of independent replication. Diversity of antibiotics resistance by plasmids and its easy spread have become so important that bacteria isolates from infections, including eye infections, are now tested for antibiotics susceptibility so that the antibiotics regime can be adjusted accordingly[2]. It has been found that the conduct and prescription policies in Health centers aid the spread of these plasmids.[3]

HIV/AIDS nearly always affect the eye and ocular signs and symptoms are the initial presenting signal that could lead to the diagnosis of HIV.[4] The New York department of Health recommends that patients with CD4 count lower than 50 cells/mm should receive eye examinations every six months including indirect Ophthalmoscopy, because HIV/AIDS has ocular complication.[5]

It is important that eye care practitioners be aware of resistant plasmid present in tears of patients diagnosed with HIV/AIDS to enable them give the best treatment regimen for any ocular infection in these patients.

MATERIALS AND METHODS

This was a cross sectional experimental study carried out in six phases from May 2008 to October 2008.

PHASE 1: Involved collection of tears samples from 100 HIV/AIDS sero-positive subjects, and 50 HIV/AIDS negative subjects. This was from the University of Benin Teaching Hospital, and the Central Hospital also in Benin City. Informed consent was obtained from the patients and ethical approval from the ethics committee of the University of Benin Teaching Hospital. The experiments were done at Lahor research institute. The swabs were taken to the laboratories within 2 hours of collection.

PHASE 2: Involved the use of various morphological and biochemical tests[6] to isolate and characterize the bacteria isolates from the tears. Bacteria isolates were cultured on blood agar, maConkey agar and nutrient agar. Pure colonies were purified

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on nutrient agar plates and then transferred to nutrient agar slants and stored at 0-4^{0}C for further characterization and identification. Tests for characterization and identification include: grams staining, catalase, coagulase, urease, oxidase, lactose, glucose and manitol tests.

PHASE 3: Involved the susceptibility testing of the bacteria isolates to multiple antibiotics to determine their resistance pattern using disc diffusion method according to Kirby-Bauer: fourteen commonly used antibiotics were used for this study. They include Tarivid (OFX)10ug, Perfloxacin (PEF) 10ug, Gentamycin (GN) 10ug, Ampiclox (APX) 30ug, Ciprofloxacin (CPX) 10ug, Strepthromycin (S) 30ug, Septrin (SXT) 30ug Erythromycin (E) 10 ug, Chloramphenicol (CH)30 ug, Sparfloxacin (SP) 10ug , Augumentin (AU) 30 ug, Zinnacef (Z) 30ug , Rocephin (R) 30ug and Amoxicillin (AM).

PHASE 4: Plasmid DNA isolation in *Pseudomonas aeruginosa* was carried out on multiple antibiotic resistant isolates by modification of rapid alkaline extraction procedures for screening of recombinant plasmid DNA by Brinboin and Doly[7] and zhou et al[8] Agarose, gel electrophoresis according Foundation of Africa Development in International Biotechnology (FADIB)[9] was carried out to resolve the extracted nucleic acid and to confirm the presence of Plasmids.
Isolation of plasmid DNA in *staphylococcus aureus* was done using TENS- Mini-Prep.

PHASE 5: Involved the curing and transformation of the multiple antibiotic resistant bacterial isolates. Plasmid curing is a process by which plasmids can be eliminated from bacteria host cells either by spontaneous reactions (UV, ionizing radiation or thymine starvation) or by induce treatment in the laboratory. Plasmid curing was according to Tomoeda et al[10].

PHASE 6: Involved testing the resistant pattern of the cured organism and recipient bacteria. Transfer of plasmid was by transformation process. Conjugation was carried out on multiple antibiotic resistant isolates that were plasmid mediated according to Wang et al[11], with *Escherichia coli* (K_{12} DHI) as the recipient. The susceptibility pattern of the E. coli strains was determined using the disk diffusion method.

RESULTS

A total of one hundred (100) HIV/AIDS sero-positive samples were used for this study. Out of this number, 37 (37%) had bacteria growth while the control group of 50 HIV negative samples had no appreciable bacteria growth. Five aerobic spore bearers (normal eye flora), which are bacillus species, were isolated from the HIV negative samples.

Staphylococcus aureus was isolated from 27 (27%) of the tear samples from the HIV/AIDS positive subjects. Three (3) pure strains of *Staphylococcus aureus* were found to be multiple antibiotic resistant (MAR). These were finally profiled, cured and transformed. Only one of the resistant strains contained plasmids.

Pseudomonas aeruginosa was isolated from 10 (10%) of the tear samples from the HIV/AIDS positive subjects and from these, four (4) pure strains of *Pseudomonas aeruginosa* were found to be multiple antibiotic resistant (MAR). These were finally profiled, cured and transformed. *E. coli* was used as recipient for transformation. None contained plasmids.

Table 1: Cultural, Morphological and Biochemical Characteristics of Bacteria isolates

Gram Staining	Catalase text	Coagulase test	Citrate text	Urease test	Oxidase test	Lactose test	Cellulose	Mannitol	
Staphylococcus aureus	Positive cocci	+	+	NA	-	NA	-	-	-
Pseudomonas aeruginosa	Negative bacilli	NA	NA	+	-	+	-	-	-

NA - Not Applicable

Table 2A: Distribution of resistant strains of *Staphylococcus aureus* before curing

Antibiotic Sample		OFX	PEF	GN	APX	Z	AM	ROG	CPX	S	SXT	E	CH	SP	AU
S_4	-	R	S^{+++}	R	S^{+++}	S^{+}	S^{++}	S^{++}	S^{++}	R	R	-	-	-	
S_{11}	-	R	S^{+}	R	S^{+}	R	S^{+}	R	S^{+}	S^{+}	R	-	-	-	
S_{19}	-	S^{+++}	S^{+++}	R	S^{++}	R	S^{+++}	S^{+++}	S^{+++}	R	R	-	-	-	

Table 2B: Distribution of resistant strains of *Pseudomonas aeruginosa* before curing

Antibiotic Sample	OFX	PEF	GN	APX	Z	AM	ROG	CPX	S	SXT	E	CH	SP	AU
P_1	**S^{+}**	-	**R**	-	-	**S^{+}**	-	**S^{++}**	**R**	**R**	-	**R**	**S^{+}**	**R**
P_8	S^{+}	S^{+}	S^{++}	-	-	R	-	S^{++}	R	S^{+}	-	S^{+}	S^{+}	R
P_9	-	-	R	-	-	S^{+}	-	S^{++}	R	R	-	R	S^{+}	R
P_{10}	S^{+}	S^{+}	S^{++}	-	-	R	-	S^{++}	R	S^{+}	-	S^{+}	S^{+}	R

KEY

S_4 - *Staphylococcus aureus*
S_{11} -
S_{19} -
P_1 - *Pseudomonas aeruginosa*
P_8
P_9
P_{10}
R - Resistant
S^{+++} - Sensitivity distance of 2.5cm S^{++} - Sensitivity distance of 2.0cm S^{+} - Sensitivity distance of 1.5cm

TABLE 3A:Resistant pattern of *staphylococcus aureus* after curing

Antibiotic Sample	OFX	PEF	GN	APX	Z	AM	ROG	CPX	S	SXT	E	CH	SP	AU
S_{7}	-	R	S^{++}	R	S^{++}	S^{++}	S^{++}	S^{++}	S^{++}	R	R	-	-	-
S_{11}	-	S^{++}	S^{+}	S^{++}	+	S^{+}	S^{+}	S^{++}	S^{+}	S^{+}	S^{+}	-	-	-
S_{19}	-	S^{+++}	S^{+++}	R	S^{++}	R	S^{+++}	S^{+++}	S^{++}	R	R	-	-	-

TABLE 3B: Resistant pattern of *Pseudomonas aeruginosa* after curing

Antibiotic Sample	OFX	PEF	GN	APX	Z	AM	ROG	CPX	S	SXT	E	CH	SP	AU
P_{1}	S^{+}	.	R	-	.	S^{+}	-	S^{++}	R	R	-	R	S^{+}	R
P_{8}	S^{+}	S^{+}	S^{++}	-	.	R	-	S^{++}	R	S^{+}	-	S^{+}	S^{+}	R
P_{9}	-	-	R	-	.	S^{+}	-	S^{++}	R	R	-	R	S^{+}	R
P_{10}	S^{+}	S^{+}	S^{++}	-	.	R	-	S^{++}	R	S^{+}	-	S^{+}	S^{+}	R

Table 4A: Resistance pattern of the recipient *E.coli* before transformation

Antibiotics	OFX	AM	GN	AX	PEF	CN	APX	Z	R	CPX	S	SXT	E
K^{1}_{12}	S	S	R	S	S	S	S	S	S	S	S	S	S

$\mathbf{K^{2}_{12}}$ – *E. coli*

Table 4B: Resistant pattern of the recipient *E.coli* after transformation

Antibiotics	OFX	AM	GN	AX	PEF	CN	APX	Z	R	CPX	S	SXT	E
K^{11}_{12}	S	S	R	S	S	S	S	S	S	S	S	S	S

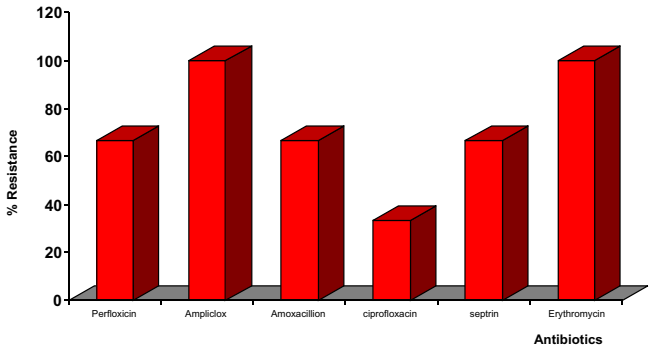

Figure 3A: Percentage Resistance of Pure *Staphylococcus aureus* Isolates Before Curing.

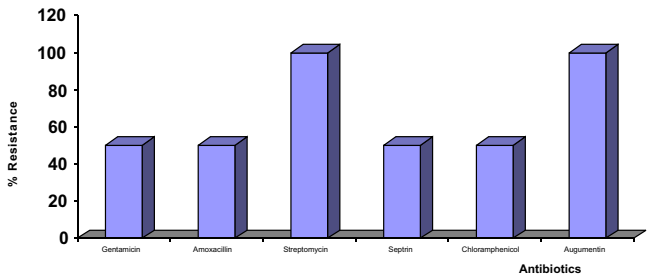

Figure 3B: Percentage Resistance of Pure *Pseudomonas aeruginosa* Isolates before curing

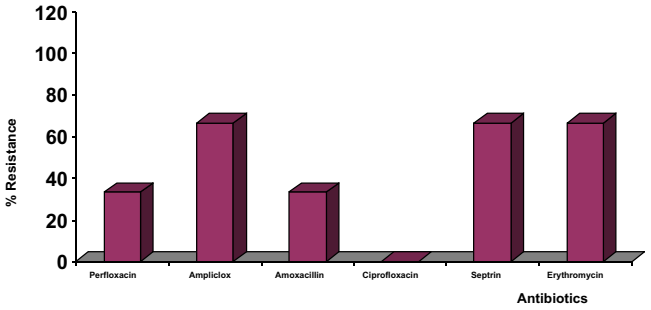

Figure 4A: Percentage Resistance of *Staphylococcus aureus* isolates after curing

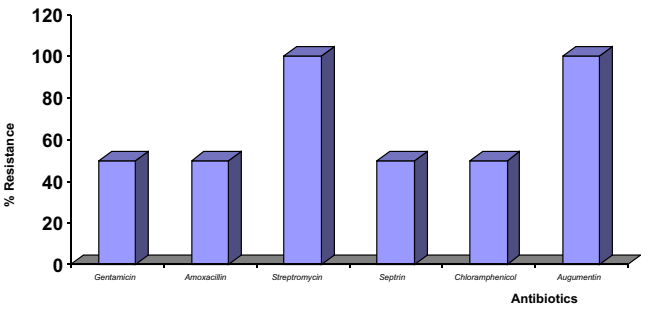

Figure 4B Percentage Resistance of *Pseudomonas aeruginosa* Isolates after curing

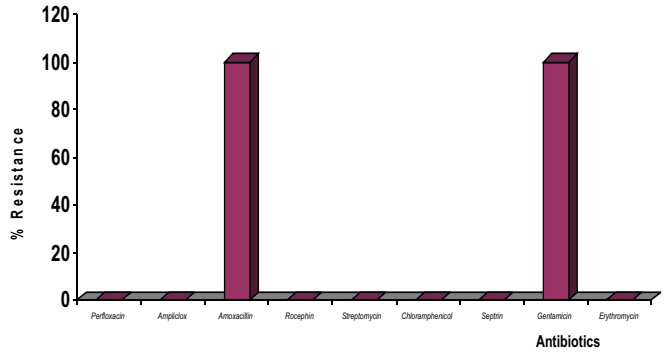

Figure 5A: Resistance pattern of recipient *E.coli* (K-12) before transformation with *Staphylococcus aureus*.

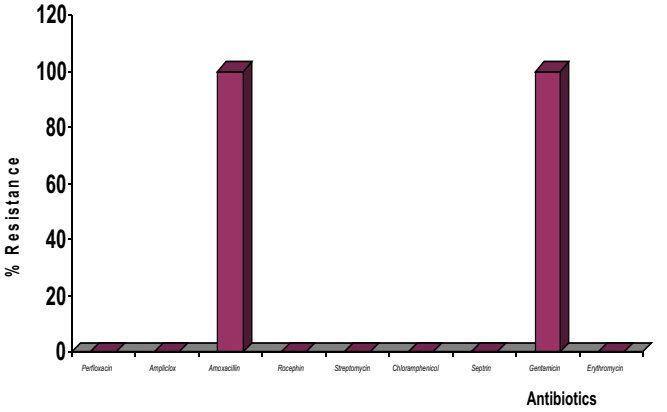

Figure 5B: Resistance pattern of recipient *E.coli* (K-12) after transformation with *Staphylococcus aureus* isolate.

DISCUSSION

Inappropriate use of antibiotics is known to play a major role in the development and spread of resistant bacteria. This is common in HIV/AIDS patients due to the increased chance of infections with opportunistic pathogens as a result of their immuno-suppressed state. They are plagued with multiple infections, necessitating the use of multiple drugs at same time. Non-compliance with regimen, abuse and disruption of the normal floral aid in the development and spread of resistance in these patient[2].

Multiple antibiotic resistances to useful classes of antibiotics including beta-lactam, aminoglycoside and quinolones have generally increased among a number of pathogens from HIV/AIDS patients[3]. The organisms under review in this study were *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from tears of HIV/AIDS positive patients. Their cultural, morphological and biochemical characteristics are as shown in Table 1.

The result of this current study showed that only 1 out of the 3 pure strains of resistant *Staphylococcus aureus* contained plasmids. This plasmid was made visible on 1% agarose gel electrophoresis. This could mean that resistance of this strain was not plasmid-mediated since the other two strains of *Staphylococcus aureus* still remained resistant. Also, there was no presence of plasmid in the resistance pattern of P*seudomonas aeruginosa* strains before and after curing, although there were multiple antibiotic resistance. This could presuppose that plasmids alone cannot cause multiple antibiotics resistance in bacteria. The fear of which poses potential health problems especially in HIV/AIDS patients with compromised immunity, as the HIV/AIDS pandemic continues to increase due to excessive use or misuse and abuse of antibiotics by HIV/AIDS patients.

Furthermore, numerous infectious pathogens that can affect the eyes may become multiple antibiotic resistant since their resistance is not solely due to plasmids. Hence, potent class of antibiotics that are rarely used in developing countries should be made available as a potential substitute to the antibiotics that currently showed a high resistance pattern.

CONCLUSION

Potent classes of antibiotic such as the quinolones that are rarely used in developing countries should be made available as a potential substitute to presently available antibiotics to which wide spread resistance have developed. Further research should be carried out on the effect of chromosomal DNA and other factors that could cause multiple antibiotic resistance among HIV/AIDS patients.

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REFERENCES

1. World Health Organization (WHO). Estimate for 2001. Death by cause, sex and mortality stratum in regions, World Health Report. Geneva 2.
2. Enebulele I. O., Ajayi O. B., Iyamu E. Susceptibility of Microorganisms Isolated from Tears of HIV/AIDS patients to Commercially Available Antibiotics. *Trop J Environ. Sci. and Hlth.* 2006: **17(1):** 01–06
3. Ling T. K., Jianhul X., Yunsong Y., Ching C. L., Hulfen Y., Peter M. H. The Antimicrobial. Susceptibility study of gram negative bacteria isolated from patients with community acquired infectious in the people republic of China. A*ntimicrobiol. ag.and chemother.* 2004: **50(1):** 374–378.
4. Sanders R., Craing E. A., France A. J., Luguhart G. H. Patients with recognized HIV infection. *Arch. Ophthalmol*. 1993; **(5):** 662–665.
5. Deepak G. Reviewing HIV/AIDS. Optometric Management. 2006; **(8):** 26–28.
6. Cown S. T. and Steel K. Isolation and Characterization of Bacteria *J. Clin. Microbial.* 1993: 134 (6)
7. Brinboin H. C., Doly H. C. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *J. Clin. Microbiol.* 1979; **(7):** 1513–1523.
8. Zhou C., Yujan Y., Ambrose Y. J. TENS- Mini-prep for isolation of plasmids. *Clin. Microbiol.* 1990; **34:** 234–240

9. Foundation for Africa Development through International Biotechnology. 2000. CPD workshop practical for gel electrophoresis.
10. Tomoeda M and Miller R. V. Plasmid curing. *J. Clin. Microbiol.* 1986; **35:** 102–105.
11. Wang M., Daniel F. S., George A. J., David C. H. Emerging plasmids mediated quinolones resistance gene associated with the qnr gene in *Klebsiella pneumonia* clinical Isolates in the United States. *Antimicrob. Ag. Chemother.* 2004; **48(4):** 1295–1299.
12. Stephen T. A. Antimicrobial therapy. *Scientific America.* 2007; **3:** 46–53.