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Evaluation of the Types of Bacteria in the Blood of HIV-1 Patients Attending ART Clinic at the FMC Owo, Nigeria and their Antibiogram Profile

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Abstract

In this investigation, the types of bacteria present in the blood of HIV-1 positive individuals attending the Antiretroviral Therapy clinic at a tertiary healthcare centre in Southwest, Nigeria and their antibiogram profile were assessed. A total number of Five Hundred confirmed HIV-1 Positive Patients were recruited for this study. Their blood was collected and subjected to standard microbiological techniques to isolate and identify the types of bacteria present and also determine the antibiogram profile of the isolates. The bacterial species identified are *Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens, Staphylococcus aureus, Staphylococcus lentus, Salmonella enterica serovar Typhimurium, Salmonella enterica serovar Typhi, Salmonella enterica serovar Enteritidis, Proteus mirabilis, Proteus vulgaris, Enterobacter aerogenes, Escherichia coli*, O103:H2, *Escherichia coli* O26:H11, *Shigella flexneri, Shigella dysenteriae, Shigella sonnei* and *Streptococcus pneumoniae*. *Escherichia coli* and *Salmonella typhimurium* were isolated from the control group of apparently healthy individuals consisting of fifty respondents. Approximately, 4% of the patients were found to have more than one type of bacterial species in their blood. The antibiogram profile of isolates revealed Ofloxacin as the most effective antibiotic against most isolated bacterial species. Only two isolates were resistant to each of Streptomycin and Chloramphenicol. Proteus mirabilis displayed the highest level of resistance while other bacterial isolates exhibited resistance to Cotrimoxazole, Augmentin, Amoxycillin, Cloxacillin and Erythromycin.

Keywords: HIV; Blood; Bacteria

Introduction

Human Immunodeficiency Virus (HIV) infection is still a serious problem globally. It is usually contracted through contact with HIV - infected blood and other body fluids via mucosal membranes or non-intact skin surfaces, sexual intercourse, intravenous drug use and vertical transmission (mother to child transmission) [1-3].

The management of HIV infection is enhanced by early detection, prompt CD_4 assessment and strict adherence to antiretroviral therapy. The scourge of HIV/AIDS is much felt in Africa, with much burden in the sub-Saharan and Southern Africa [4].

Bacteraemia is a major cause of morbidity and mortality in HIV Patients. This is because in addition to the pathological effects caused by these microorganisms, the immunological response elicited by the immune system of the host to these microorganisms in the bloodstream can lead to septic shock, which usually has high mortality rate [5].

Although infections caused by bacterial pathogens can be treated with antibiotics, however the issue of their resistance to most of the available antibiotics makes this difficult. Some of the bacterial species implicated in bloodstream infections are *Klebsiella* sp. and *Escherichia coli* [6]. This study therefore is to screen the blood of HIV-1 patients in the studied community for the types of bacterial pathogens present and to know the antibiogram profile of these microorganisms.

Materials and Methods

Patients were recruited from the HIV Clinic of the Federal Medical Centre, Owo, Ondo State, Nigeria. The sampling and isolation processes with suitable bacteriological media were commenced in May, 2015.

A total of Five Hundred (500) serologically confirmed HIV-1 patients, who were previously identified through the national parallel algorithm for HIV testing provided by the United State Agency for International Development (USAID) were recruited for the study, consisting of 162 males and 338 females across all age groups, 452 patients under Antiretroviral Therapy and 48 Non - Antiretroviral Therapy Patients.

Retroviral screening is conducted using the the National serial algorithm for HIV testing. The algorithm consists of Determine - Unigold - Stat pak (tiebreaker).

The blood samples (non-repeat) were collected after approval by the ethical review committee of the healthcare institution.

Two milliliters (2 ml) of the collected blood was introduced into 5ml Brain-Heart infusion broth and were incubated at 37°C for 24hrs before culturing on suitable agar plates for bacterial growth [7]. The bacterial isolates from each plate were subcultured for pure colonies at 37°C for 18 - 24 hours. The isolates were identified by Gram staining, biochemical tests, sugar fermentation, motility test and molecular characterization techniques.

The antibiotics used were manufactured by Abtek Biologicals Limited, Liverpool, United Kingdom. They comprised both broad spectrum and narrow spectrum antibiotics engaged in the treatment

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of both Gram positive and Gram negative bacterial infections. The antibiotics are Cotrimoxazole (25 µg), Cloxacillin (5 µg), Erythromycin (5 µg), Gentamicin (10 µg), Augmentin (30 µg), Streptomycin (10 µg), Tetracycline (10 µg), Chloramphenicol (10 µg), Ofloxacin (5 µg), Nalidixic Acid (30 µg), Nitrofurantoin (200 µg), Amoxycillin (25 µg).

The antibiotic sensitivity testing of isolates was done employing the Kirby Bauer's method, Mueller Hinton agar was prepared according to manufacturer's specification and about 15 ml was poured into sterile Petri dishes at about 45°C. Fresh cultures of about 18 hours of each isolate was then used to seed the agar, after obtaining a broth culture compared with 0.5 Mcfarland's standard solution. The antibiotic discs for testing were then placed on the surface of each seeded agar medium. These were incubated for 24 hours at 37°C. Sensitvity was recorded for any isolate that had a clear zone of inhibition of up to 17 mm diameter or more for each disc [8].

The DNA extraction, amplification and gel electrophoresis of amplicons were conducted by adopting the method of Barraquio et al. [9].

The DNA from bacterial isolates were extracted and purified using the Qiagen Extraction Kit, samples from, an aliquot portion of a previously 24-hrs broth culture made from each isolate was aseptically transferred into each kit and vortexed for about 15 minutes. The cells broke up, liberating the DNA of each bacterial isolate.

The DNA extracted from the bacterial cells were amplified with the aid of the thermocycler (PCR machine), using the primers - 1101F [(16S r RNA forward) 5'–AAC GAG CGC AAC CC – 3'] and 1407 R [(16S rRNA reverse) 5'–GAC GGG CGG TGT GTA C–3'] universal primers. The DNA pellet was added with 30 µL Tris Edetate (TE) buffer, then incubated at 65°C for 15 min. The stages in the amplification included initial denaturation (94oC for 5 minutes), cycling, denaturation 94°C for 1 minute, annealing (52°C for 30 secs), extension (72°C for 8 minutes) and holding (4°C for 20 minutes). The DNA polymerase used was Taq polymerase.

A total of 5 µL of the DNA isolate were mixed with 3 µL violet buffer on paper film, and then the mixture was electrophoresed at 5 v cm using 1.5% agarose gel in Tris-acetate EDTA (TAE) buffer. Then the purity of the DNA isolated was visualized under UV light, after staining for 30 min with 0.2 mg mL ethidium bromide adopting the method of Barraquio et al. [9].

The amplicons were sequenced, the nucleotide sequences were compared with the standard reference sequences for alignment using the Basic Local Alignment Search tool (BLAST) from the National Centre for Biotechnology Information (NCBI).

Data analysis and interpretation of results

Statistical Package for Social Sciences Version 17.0 (SPSS Inc., Chicago, IL) was used for all analyses. Descriptive statistics were used in the computing of the results in order to give lucid representations of the data analysed. The Chi-square (X^2) test was used to determine significant differences and effects.

Results

Types of bacteria isolated

A total of ten (10) bacterial species were isolated from the blood of HIV-1 Patients. These bacterial isolates are *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Shigella dysenteriae, Proteus mirabilis* and *Enterobacter* sp. (Table 1). The frequency of occurrence of these bacterial species from this investigation can be seen in (Table 1). More than one bacterial species were isolated in 22 of the patients sampled as seen in Table 2.

S/No	Probable Organism	Frequency (%).
1	*Escherichia coli*	29(10).
2	*Pseudomonas aeruginosa*	35(12).
3	*Salmonella typhimurium*	23(8).
4	*Shigella dysenteriae*	57(20).
5	*Staphylococcus aureus*	26(9).
6	*Klebsiella pneumoniae*	56(19).
7	*Salmonella typhi*	15(5).
8	*Streptococcus pneumoniae*	22(8).
9	*Enterobacter aerogenes*	17(6).
10	*Proteus mirabilis*	13(4).
Total	**10**	**293**

Table 1: Frequency of occurrence of bacterial isolates from the blood samples of HIV-1 Patients.

S/No	Bacterial isolates	Frequency (%)
1.	*Staphylococcus aureus* / *Klebsiella pneumoniae*.	10(45%)
2.	*Escherichia coli* / *Salmonella typhimurium*	2(9%)
3.	*Escherichia coli* / *Shigella dysenteriae*.	5(23%)
4.	*Proteus mirabilis* / *Salmonella typhimurium*.	5(23%)
	Total	**22**

Table 2: Frequency of occurrence of mixed bacterial pathogens in the blood samples of HIV-1 patients examined.

Using the 16 subunit ribosomal DNA technique [9], the following bacterial species were sequenced, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Staphylococcus aureus, Staphylococcus lentus, Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Typhi, *Salmonella enterica* serovar Enteritidis, *Proteus mirabilis, Proteus vulgaris, Enterobacter aerogenes, Escherichia coli,* O103:H2, *Escherichia coli,* O26:H11, *Shigella flexneri*, *Shigella dysenteriae* and *Shigella sonnei*.

Seventy-Eight (78%) percent of cases were by sexual transmission, 19.8 percent were by vertical transmission, 2.2 percent of cases were from contact with blood other than sexual transmission (parenteral route).

The overall evaluation of the CD_4 counts of HIV-1 Patients studied revealed that more of the female patients had their CD_4 counts within the normal CD_4 range than their male counterparts.

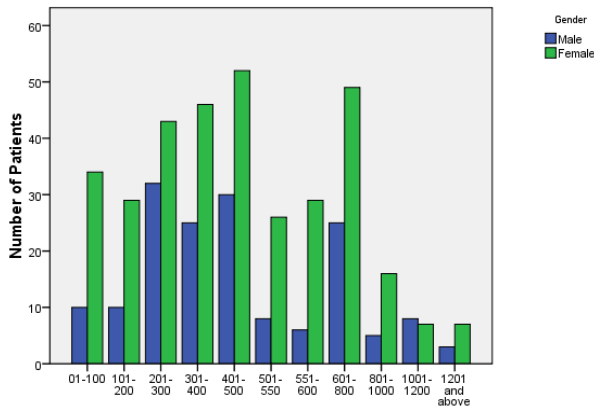

Figure 1: The categorization of Patients' CD_4 Counts according to their gender. Result is statistically significant (P = 0.015).

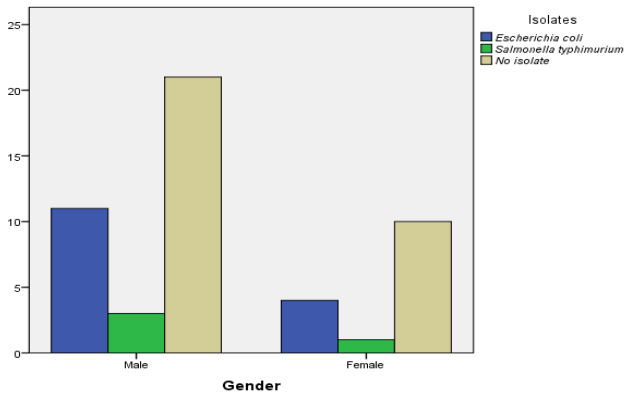

Figure 2: The distribution of respondents in the control group according to their gender and occurrence of bacterial isolates. Result is not statistically significant (P = 0.124).

This suggests better immune reconstitution in the group of female patients studied (Figure 1).

There was a minimal level of occurrence of microbial isolates in the control group, the bacterial isolates implicated in the respondents were *Escherichia coli* (30%) and *Salmonella typhimurium* (8%). Over 60% of respondents were found to have no bacterial isolates in their bloodstream (Figure 2).

Antibiotic sensitivity profile of the bacterial isolates from HIV-1 Patients sampled

Ofloxacin and Gentamicin were found with the highest antimicrobial activities while Cloxacillin was found to have the least antibacterial effect on the bacterial isolates. Nalidixic acid, Nitrofurantoin, Tetracycline, Streptomycin and Chloramphenicol had intermediate antimicrobial actions on most bacterial isolates, while bacterial isolates exhibited resistance to the following antibiotics; Cotrimoxazole, Amoxycillin, Augmentin, Cloxacillin and Erythromycin (Table 3).

Klebsiella pneumoniae displayed the highest susceptibility pattern towards tested antibiotics while *Proteus mirabilis* displayed the highest level of resistance to antibiotics tested as presented in Table 3.

Discussion

The bulk of microbial isolates from this study are members of the Enterobacteriaceae family, which is the largest and most heterogenous group of medically significant gram-negative helix-shaped bacteria. They are most frequently isolated in clinical samples. The infections include diarrhoea, dysentery, Salmonellosis, Haemolytic-uremic syndrome (HUS), necrotizing enterocolitis and various nosocomial infections. Pathogenicity of Enterobacteriaceae as a family of gram negative bacteria is associated with the Lipopolysaccharide (LPS) situated in the outer membrane of the bacterial cell wall which is usually responsible for endotoxin production in Gram negative bacteria – a cause of septicemia. Infections from enterobacteriaceae are regarded as one of the two leading killers of children in developing countries. This goes in agreement with the submission of Frey and Sherk [10].

Shigella species were the most commonly encountered isolates in the research, this could be due to negligence of patients in ensuring proper hygiene with regards to food preparation, handling, their choices of food vendours and/or ineffective feeding habits (malnutrition). This is also an indicator to the increasing rate of Shigella bacteraemia. According to the findings of Trevett et al. [11], the presence of Shigella bacteraemia occurs usually in adults with underlying disease conditions, such as leukaemia, diabetes, sickle cell anaemia, cirrhosis and HIV. Kotloff et al. [12] also submitted that HIV infected individuals are at high risk of recurrent, severe and fatal occurrences of Shigella bacteremia.

The most frequent occurrence of mixed bacterial populations in the samples was found to occur between *Staphylococcus aureus* and *Klebsiella pneumoniae* category. Both of these bacteria commonly colonise the upper respiratory tract, which is a part of the body notable for significance in pathogenesis of infections and epidemiology. This is in line with a report from Bodenstein and Du Toit [13] on the evaluation of the susceptibility of these microorganisms to selected classes of flavonoids.

Ofloxacin, which is a member of the Quinolone antibiotics displayed the highest antimicrobial effect of all the antibiotics used in the antimicrobial susceptibility testing, this outcome agrees with a report from Prajna et al. [14] on the bacteriologic and clinical efficacy of Ofloxacin and Ciprofloxacin Opthalmic solutions in the treatment of Bacterial Keratitis, stating that the antibiotics are effective and safe in the treatment of patients with culture positive bacterial keratitis. However, Multum [15] reported major side effects on ofloxacin are onset of pseudomembraneous colitis during or after treatment, seizures, diarrhea, easy bruising or bleeding, pale skin, severe skin reactions and fever. *Proteus mirabilis* was the most resistant microorganism to antimicrobial testing. This is likely due to the mutation rate of the particular species of Proteus, that confers antibiotic resistance to it. This agrees with the report of Feglo et al. [16], that *Proteus* spp. is resistant to Ampicillin, Cotrimoxazole, Tetracycline and Chloramphenicol. A report from Newmann et al. [17] further buttresses this phenomenon.

Conclusion

This study has been able to show that *Shigella dysenteriae* is the most frequently encountered bacterial species in the blood of HIV-1 patients in the community sampled while the least encountered

	Bacterial Isolates	GEN	NAL	NIT	COT	AMX	TET	AUG	OFL	CXC	STR	ERY	CHL
1	*Escherichia coli*	14	37	37	86	96	27	100	12	100	0	80	0
2	*Klebsiella pneumoniae*	5	10	6	56	65	16	63	12	100	0	60	20
3	*Salmonella typhi*	20	7	21	87	100	14	100	7	100	100	100	50
4	*Salmonella typhimurium*	14	29	33	83	81	36	91	14	100	0	100	50
5	*Staphylococcus aureus*	14	0	20	76	60	8	87	0	75	14	88	32
6	*Streptococcus pneumoniae*	5	22	11	67	56	10	65	22	100	0	91	0
7	*Pseudomonas aeruginosa*	10	0	0	80	92	25	86	0	64	40	50	36
8	*Shigella dysenteriae*	11	36	19	66	69	18	62	7	75	27	54	27
9	*Proteus mirabilis*	23	31	23	100	100	23	100	8	100	100	100	100
10	*Enterobacter aerogenes*	6	6	0	82	87	19	75	0	100	0	100	100

Table 3: The Resistance Pattern of Bacterial isolates to antibiotics (%).

KEY: GEN – GENTAMICIN (10 µg). NAL - NALIDIXIC ACID (30 µg). NIT – NITROFURANTOIN (200 µg). COT - COTRIMOXAZOLE (25 µg). AMX – AMOXYCILLIN (25 µg). TET – TETRACYCLINE (10 µg). AUG - AUGMENTIN (30 µg). OFL - OFLOXACIN (5 µg). CXC – CLOXACILLIN (5 µg). STR – STREPTOMYCIN (10 µg). ERY - ERYTHROMYCIN (5 µg), CHL – CHLORAMPHENICOL (10 µg).

pathogen was *Proteus mirabilis.* All the bacterial species isolated are resistant to Cloxacillin, Cotrimoxazole, Amoxycillin, Augmentin and Erythromycin but sensitive to Ofloxacin [18-24].

References

1. De Cock KM, Fowler MG, Mercier E, de Vincenzi I, Saba J, et al. (2000) Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. JAMA 283: 1175-82.
2. Cohen MS, Gay CL (2010) Treatment to prevent transmission of HIV-1. Clin Infect Dis 3: S85-95.
3. Ward H, Rönn M (2010) Contribution of sexually transmitted infections to the sexual transmission of HIV. Curr Opin HIV AIDS 5: 305-10.
4. Gillespie S, Kadiyala S (2005) HIV/AIDS and food and nutrition security: from evidence to action. Washington DC: International Food Policy Research Institute.
5. Ramachandran G (2014) Gram-positive and Gram-negative bacterial toxins in sepsis. Virulence 5: 213-218.
6. Yinnon AM, Butnaru A, Raveh D, Jerassy Z, Rudensky B (1996) Klebsiella bacteraemia: community versus nosocomial infection. QJM 89: 933-941.
7. Clinical and Laboratory Standards Institute (2007) Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard – Sixth Edition. CLSI document H3-A6. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Pennsylvania 19087-1898 USA.
8. Hudzicki J (2013) Kirby – Bauer disk diffusion susceptibility test protocol. American Society for Microbiology Microbe Library.
9. Barraquio VL, Djoko K, Elegado FB (2008) Phenotypic and Genotypic Characterization of Probiotic Bacteria Isolated from Probiotic Dairy Products in the Philippines. Philippine Journal of Science 137: 77-83.
10. Frey R, Sherk S (2006) Gale Encyclopaedia of Children's Health: Infancy through Adolescence.
11. Trevett AJ, Ogunbanjo BO, Naraqi S, Igo JD (1993) Shigella bacteraemia in adults. Postgrad Med J 69: 466-468.
12. Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, et al. (1999) Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. Bulletin of the world Health Organisaion 77: 651-666.
13. Bodenstein J, Du Toit K (2012) The Susceptibility of Staphylococcus aureus and Klebsiella pneumoniae to Naturally Derived Selected Classes of Flavonoids.
14. Prajna NV, George C, Srinivasan M, Lu KL, McDonnell PJ (2001) Bacteriologic and Clinical Efficacy of Ofloxacin 0.3% Versus Ciprofloxacin 0.3% Opthalmic Solutions in the Treatment of Patients with Culture-positive Bacterial Keratitis. Cornea 20: 175-178.
15. Multum C (2011) Ofloxacin (oral) Consumer Information.
16. Feglo PK, Gbedema SY, Quay SNA, Adu-Sakordie Y, Opoku-Okara C (2010) Occurrence, species distribution and antibiotic resistance of Proteus isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. International Journal of Pharma Sciences and Research (IJPSR) 1: 347-352.
17. Newmann MJ, Frimpong E, Asamoah –Adu A, Sampane-Donkor (2006) Resistance to Antimicrobial Drugs in Ghana. The Ghanaian – Dutch collaboration for Health Research and Development pp. 1-6.
18. Cohen S, Janicki-Deverts D, Miller GE (2007) Psychological Stress and Disease (HIV/AIDS). JAMA 298: 1685-1687.
19. Ernst E (2005) Regulation of complementary and alternative medicine. Focus on Alternative and Complementary Therapies.
20. Mahan LK, Escott-Stump S (2007) Krause's food & Nutrition therapy.
21. Ministry of Health and Social Welfare (2007) National Health Policy. Mbabane: Government of the Kingdom of Swaziland.
22. Philpott DJ, Edgeworth JD, Sansonetti PJ (2000) The Pathogenesis of Shigella flexneri infection: Lessons from in vitro and in vivo studies. Philos Trans R Soc Lond B Biol Sci 355: 575-586.
23. United States Department of Health and Human Services (2010) Opportunistic infections. Opportunistic infections and their relationship to HIV/AIDS.
24. World Health Organisation (2005) Interim WHO Clinical Staging of HIV/AIDS Case definitions for surveillance, African region.

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