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MICROBIOLOGICAL EVALUATION OF THE POTENCIES OF BRANDS OF FOUR PARENTERAL ANTIBIOTIC PREPARATIONS USED IN THE TREATMENT OF URINARY TRACT INFECTIONS

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RUNNING TITLE: POTENCIES OF PARENTERAL ANTIBIOTIC PREPARATIONS

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ABSTRACT

Urinary tract infection (UTI) is a common disease and sometimes life threatening if not properly treated. In Nigeria, aside adulteration and counterfeiting of antibiotics, potency of antibiotics can also be altered by factors like production errors and storage condition at the Pharmacy stores. This study investigated the potencies of selected brands of four common parenteral antibiotic preparations, in Nigerian drug markets against uropathogens isolated from patients with recurrent UTI.

Ten selected clinical bacterial isolates from patients with recurrent UTI were collected from the Microbiology unit of the University College Hospital, Ibadan and authenticated by standard bacteriological methods. The isolates were subjected to susceptibility test against eight standard antibiotics by disc diffusion method. The selected brands of the four parenteral antibiotic preparations used in this study includes: Ciprofloxacin (Emason® and Uniflox®); Ceftriaxone (Rocephin® and Cefin®); Aminoglycoside (Pe-genta® and Philo-genta®) and Aminopenicillin/inhibitor (Augmentin® and Amoxiclav®). Efficacies of the parenteral antibiotic preparations against the isolates were determined by Minimum Inhibitory Concentrations (MICs) using broth-dilution method.

Antibiotic susceptibility test using standard antibiotic discs showed that all (100%) the bacterial isolates were multidrug resistant (MDR), being resistant to two or more classes of antibiotics. Aside *E. coli* (E1) that was susceptible to the two brands of gentamicin preparations at the Clinical Laboratory Standard Institute (CLSI) susceptibility breakpoint (≤ 4 µg/mL), all the other isolates showed resistance to the four parenteral antibiotic preparations and were only susceptible at higher concentrations (> 2 folds) above the CLSI resistance breakpoints for the different antibiotic preparations. The brands of the parenteral antibiotic preparations used in this study have low potency which varies with different bacterial strains involved.

L'EVALUATION MICROBIOLOGIQUE DES PUISSANCES DES MARQUES DE QUATRES PREPARATIONS ANTIBIOTIQUES PARENTERALES UTILISEES DANS LE TRAITEMENT DE L'INFECTION DES VOIES URINAIRES.

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RESUME

L'infection des voies urinaires (UTI) est une maladie commune et parfois met la vie en danger si pas correctement traitée. Au Nigeria, a part de la falsification et la contrefaçon des antibiotiques, la puissance des antibiotiques peut également être modifiée par des facteurs tels que les erreurs de production et les conditions de stockage dans les magasins de la pharmacie. Cette étude a examiné les puissances des marques sélectionnées de quatre préparations antibiotiques parentérales courantes, aux marchés nigérians de la drogue, contre les uropathogenes isolées des patients avec infection urinaire récidivante.

Dix isolats bactériens sélectionnés des patients avec infection urinaire(UTI) récidivante ont été recueillies de l'unité microbiologie de l'hôpital Universitaire, Ibadan et authentifie par des méthodes bactériologiques classiques. Les isolats ont été soumis à des tests de sensibilité contre huit antibiotiques Standards par la méthode de diffusion sur disques. Les marques sélectionnées de quatre préparations antibiotiques parentérales utilisées dans cette étude comprennent : Ciprofloxacine (Emason® et Uniflox®) ; Ceftriaxone (Rocephine® et Cefin®) ; Aminoside (Pe - genta® et Philo – genta®) ; et Aminopenicilline/inhibiteur (Augmentin® et Amoxiclav®). L'efficace des préparations antibiotiques parentérales contre les isolats ont été déterminés par concentrations minimales inhibitrices(MICs) en utilisant la méthode du bouillon – dilution.

Le test de sensibilité aux antibiotiques en utilisant les disques antibiotiques standard a montré que tous (100%) les isolats bactériens étaient multi résistants (MDR), étant résistant aux deux ou plusieurs classes d'antibiotiques. A part de *E.coli* (E1) qui était sensible aux deux marques de préparations gentamicine a l'Institut de Laboratoire Clinique Standard. Le point d'arrêt de la sensibilité (≤ 4µg/ml) tous les autres isolats ont montré résistance aux quatre préparations antibiotiques parentérales et étaient seulement sensibles a des concentrations plus élevées (> 2 plis) au- dessus des points d'arrêt de résistance CLSI pour les préparations antibiotiques différentes. Les marques des préparations antibiotiques parentérales utilisées dans cette étude ont une faible puissance qui varie avec les souches bactériennes différentes impliquées.

INTRODUCTION

Urinary system is classified into Lower (urethra, bladder) and upper (kidneys, renal pelvis) urinary tract, and the infection of the urinary system is when any one or all parts of the urinary system are infected by microorganisms, mostly bacteria, with significant bacteriuria in the presence of symptoms (1). Urinary tract infection (UTI) is described based on the location of the infection in the urinary system as either lower urinary tract infection (LUTI) or upper urinary tract infection (UUTI). Usually, someone is declared to have urinary tract infection (UTI) only if repeated viable count of the microorganisms in the urine samples of the person concern is $\geq 10^5$ $CFUmL^{-1}$ of the urine (1).

Urinary tract infection caused by bacterial isolates is of global concern and the major setback in its treatment using antibiotic is the emergent of highly resistant bacterial strains (2, 3). Common bacterial isolates usually involved in UTI, either uncomplicated or complicated, are *Escherichia coli*, which is the most common, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Streptococcus saprophyticus*, species of *Enterobacter*, *Serratia*, *Acinetobacter*, and *Pseudomonas aeruginosa* (3, 4, 5, 6). Aside the first line antibiotics such as trimethoprim-sulphamethoxazole, penicillins and nitrofurantoin, which are now obsolete in the treatment of UTI due to high level of resistance (7, 8), three major classes of antibiotics commonly used as second line treatment of UTI are the fluoroquinolones, aminoglycosides and some beta-lactams (9). Resistance to these classes of antibiotics have been reported world-wide including Nigeria by several authors in their laboratory screening of clinical uropathogens and has contributed immensely to the problems encountered by physicians in the treatment of UTI (10, 11). Taken of antibiotics for too short a time, at an inadequate concentration, or for the wrong treatment, all constitute irrational use of antibiotics and thus contribute to the development of resistance among clinical bacterial isolates (2, 12, 13). Another issue of major concern is the use of antibiotics as growth promoters in food-producing animals and poultry flocks (14). Such practices have contributed to the rise in the level of antibiotic resistance and dissemination of resistance traits among microorganisms which in turns can be transmitted from animals to humans (15, 16, 17, 18). This study however, evaluated the potencies of two brands each, of fluoroquinolone (ciprofloxacin), aminoglycoside (gentamicin), cephalosporin (ceftriaxone) and amino-penicillin/inhibitor combination (amoxicillin-clavulanic acid) obtained from local pharmaceutical market in Ibadan against selected multidrug resistant uropathogenic bacterial isolates from patients diagnosed with recurrent UTI.

MATERIALS AND METHODS

COLLECTION OF UROPATHOGENIC MICROORGANISMS

Ten bacteria isolated from ten patients with recurrent urinary tract infection were collected from the Microbiology and Parasitology Department of the University College Hospital (UCH), Ibadan on sterile nutrient agar slants and were authenticated by standard bacteriological techniques. Pure cultures of the authenticated bacterial isolates were sub-cultured on fresh nutrient agar slants and stored in the refrigerator at 4°C. The bacterial isolates include two strains each of *Pseudomonas aeruginosa* (Ps1 & Ps2), *Staphylococcus aureus* (S1 & S2), *Proteus spp* (Pr1 & Pr2), *Klebsiella spp* (K1 & K2), and *Escherichia coli* (E1 & E2).

SUSCEPTIBILITY TEST USING STANDARD ANTIBIOTIC DISCS

Antibiotic sensitivity testing was carried out by the disc diffusion method on Mueller Hinton agar using the following selected standard antibiotic discs: amoxicillin-Clavulanic acid (AMC)- 20/10 µg, cefuroxime (CRX)- 30 µg, cefixime (CXM)- 5 µg, ceftazidime (CAZ)- 30 µg, gentamicin (GEN)- 10 µg, ofloxacin (OFX) - 5 µg, ciprofloxacin (CPR)- 5 µg, nitrofurantoin (NIT)- 300 µg. Pure colonies of each test organism were inoculated into tubes containing 10ml of sterile nutrient broth and incubated at 37°C

for 24hrs. Thereafter, a 10^{-2} dilution of the stock bacterial suspension was made and a sterile cotton swab was used to evenly inoculate the entire dried surface of previously prepared and set Mueller Hinton agar plates. The selected standard antibiotic discs were firmly placed on the set agar plates using sterilized forceps. The agar plates were left for about 30 minutes for effective diffusion of the antibiotics before being incubated at 37°C for 24 hours. The diameters of the zone of growth inhibition were measured to the nearest millimetre and the results interpreted as sensitive or resistant based on the Clinical and Laboratory Standard Institute (CLSI) 2011 guideline (19).

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION OF THE SELECTED PARENTERAL ANTIBIOTIC PREPARATIONS

The parenteral antibiotic preparations used in this study include: Aminoglycoside (gentamicin inj: Philo-genta® and Pe-genta®), Floroquinolone (ciprofloxacin inj: Uniflox® and Emason®), Cephalosporins (ceftriaxone inj: Rocephin® and Cefin®) and Amino-penicillin (amoxicillin-clavulanic acid: Augmentin® and Amoxiclav®). These antibiotics were bought from reputable pharmaceutical stores located within Ibadan.

Stock preparations of the antibiotic under investigation were diluted serially with nutrient broth such that the concentration was halved in each container in a series to give ten concentrations. This was done by adding 5ml of the solution of the test antibiotic aseptically to 5ml of double strength medium and mixed by shaking. With a fresh pipette, 5ml of the mixture was transferred aseptically to the second tube which contains 5ml single strength medium. This was also mixed by shaking and the procedure repeated until the last tube giving the following concentrations: 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0.5µg/mL. Thereafter, 0.1ml of a 10^{-2} dilution of the overnight broth culture was added to each tube. A tube containing sterile broth only served as a control. The tubes were incubated at 37°C for 24 hours and the minimum inhibitory concentrations (MICs) of the different parenteral antibiotics preparations were determined and result tabulated.

RESULTS

The microbiological characterisation of the isolates confirmed their identities to be: *Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Table 1). The results of the antibiotic susceptibility test using standard antibiotic discs shows that 90% of them were resistant to cefixime, cefuroxime, and ceftazidime. Percentage resistance to nitrofuratoin and ofloxacin was 70%, Gentamicin was 60% while for ciprofloxacin it was 20%. Seven (70%) of the isolates showed resistance to three classes of antibiotics while K2 and Pr2 each showed resistance to five and four classes respectively (Table 1). The results of the susceptibility test using different concentrations of the parenteral antibiotics are shown in table 2. All the isolates showed resistance to the four parenteral antibiotic preparations giving MIC values greater than the Clinical Laboratory Standard Institute (CLSI) guideline Resistance breakpoints for each antibiotic except against *E. coli* (E1) that was susceptible to the two brands of gentamicin preparations at 4µg/mL which was within CLSI susceptibility breakpoint of ≤4µg/mL. However, variations in potency exist among some of the different brands of antibiotics used in this study against the clinical isolates. The MIC of Uniflox® brand (4µg/mL) of ciprofloxacin against *Pseudomonas earuginosa* -Ps1 was reduced fourfold compared to that of Emason® brand (16µg/mL) as well as between Pe-gena® brand (64µg/mL) of Gentamicin and Philo-genta® brand (256µg/mL) against Ps1. Also, MIC of Uniflox® brand (64µg/mL) against *Klebsiella pneumoniae* K1 was increased fourfold compared to the Emason® brand (16µg/mL). This also occurred between Augmentin® brand (64µg/mL) of amoxicillin-clavulanic acid and Amoxiclav® brand (256µg/mL) against *Staphylococcus aureus* S1.

TABLE 1: ANTIBIOTIC RESISTANCE PROFILES OF THE UROPATHOGENS TO THE STANDARD ANTIBIOTIC DISCS

Isolate ID	Antibiotic Resistance Profile	No. of Antibiotic classes
E1	AMC, CRX, CXM, CAZ	2
E2	GEN,AMC,CRX, CXM, CAZ	3
S1	OFX, CPR, GEN,AMC	3
S2	AMC, NIT, CRX, CXM, CAZ	3
Ps1	AMC, NIT, CRX, CXM	3
Ps2	AMC, NIT, CRX, CXM,	3
Pr1	AMC, NIT, CRX, CXM,CAZ	3
Pr2	AMC, NIT, GEN, CRX, CXM, CAZ,	4
K1	AMC, GEN, CRX, CXM, CAZ	3
K2	AMC, NIT, GEN, CRX, CXM, CAZ, OFX	5

ID = Identity; E1& E2 = *Escherichia coli*, S1& S2 = *Staphylococcus aureus;* Ps1& Ps2 = *Pseudomonas aeruginosa*; Pr1& Pr2 = *Proteus mirabilis*; K1& K2 = *Klebsiella pneumoniae*, AMC = amoxicillin-Clavulanic acid; CRX = cefuroxime; CXM = cefixime; CAZ = ceftazidime; GEN = gentamicin; OFX = ofloxacin; CPR = ciprofloxacin; NIT = nitrofurantoin.

TABLE 2: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MICs) OF PARENTERAL ANTIBIOTIC PREPARATIONS BY BROTH-DILUTION

Isolate ID	Brands of Ciprofloxacin CLSI BP = ≥ 4µg/mL		Brands of Ceftriaxone CLSI BP = ≥ 4µg/mL		Brands of Amoxicillin/clavulanic acid CLSI BP = ≥ 32/16µg/mL		Brands of Gentamicin CLSI BP = ≥ 16µg/mL	
	Emason® Conc. µg/mL	Uniflox® Conc. µg/mL	Rocephin® Conc. µg/mL	Cefin® Conc. µg/mL	Augmentin® Conc. µg/mL	Amoxiclav® Conc. µg/mL	Philo-genta® Conc. µg/mL	Pe-gena® Conc. µg/mL
E1	4	4	> 256	> 256	256	256	4	4
E2	128	128	> 256	> 256	256	256	256	> 256
PS1	16	4	64	64	> 256	> 256	256	64
PS2	16	16	64	64	> 256	> 256	64	64
Pr1	8	8	64	64	128	256	64	64
Pr2	128	128	256	256	256	256	> 256	> 256
K1	16	64	> 256	> 256	256	256	> 256	> 256
K2	128	128	> 256	> 256	256	256	> 256	> 256
S1	16	16	64	64	64	256	> 256	> 256
S2	128	128	128	128	> 256	> 256	> 256	> 256

ID = Identity; E1& E2 = *Escherichia coli*, S1& S2 = *Staphylococcus aureus;* Ps1& Ps2 = *Pseudomonas aeruginosa*; Pr1& Pr2 = *Proteus mirabilis*; K1& K2 = *Klebsiella pneumoniae*, CLSI BP = Clinical Laboratory Standard Institute Breakpoint.

DISCUSSION

Antibiotic resistance is a growing problem in the treatment of infections which has led to the narrowing of antibiotic options needed to treat

bacterial infections thus making this problem a global concern and thus requiring global solution (2). Although the natural phenomenon by which resistance emerges is accelerated and amplified by a variety of factors, the most important cause is the inappropriate use of antimicrobial agents (15). With reference to the Clinical and Laboratory Standard Institute (CLSI), 2011 guidelines (19), the results obtained from the sensitivity test using standard antibiotic disc showed that all the bacterial isolates used in this study are multidrug resistant strains. This confirms the earlier report by Dada and Muili (2010) of wide spread of resistant uropathogens among patients with UTI in Ibadan, Southwest Nigeria (20).

Variation observed in the potency of some brands of the parenteral antibiotics used in this study against the same organism could be as a result of differences in the formulation methods and excipients used which may affect the penetration of the antibiotics into the bacteria cell. Also, it could be as a result of the condition of storage which could affect product potency, efficacy and overall quality. The 80% susceptibility of the isolates to the standard ciprofloxacin disc and the 100% resistance of the isolates to the two brands of ciprofloxacin injection used in this study suggest that the potency and efficacy of the parenteral antibiotic preparations have been altered either during formulation or on shelf. Therefore, stringent regulations must be put in place to ensure that formulation and storage of antibiotics is done appropriately according to standard. In the preparation of parenteral antibiotics, care must be taken to ensure that current good manufacturing practices (CGMPs) are followed so as to produce drugs of high standard that will be physically and chemically stable throughout their intended shelf life. Packaging of drugs must maintain the products' integrity throughout the shelf life and during administration. Agencies involved in the regulation of sales of drugs should make sure that pharmacies and chemists display and store their drug items under correct storage conditions without any compromise as poorly stored drugs can loss their efficacy and potency, especially antibiotics that require strict storage conditions.

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CONFLICT OF INTEREST: None to declare

ETHICAL APPROVAL: Not required

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