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Trimethoprim/sulphamethoxazole resistance in *Escherichia coli* and *Klebsiella* spp. urinary isolates

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Summary

The susceptibility of 40 *Escherichia coli* and 35 *Klebsiella* spp. urinary isolates to trimethoprim/sulphamethoxazole (cotrimoxazole) was determined. The determination was based on the activity of the standard multodiscs-cotrimoxazole (25 µg), (Oxoid); sulphafurazole (500 µg), (Mastring-S); and the minimum inhibitory concentration of trimethoprim and sulphamethoxazole singly against the isolates. Thirty-two (80%) of 40 isolates of *Escherichia coli*, and 26 (74%) of 35 isolates of *Klebsiella* spp. were resistant to cotrimoxazole. All the isolates were resistant to more than 500 mg/l sulphamethoxazole. Twenty of the 32 (62%) cotrimoxazole-resistant *E. coli* strains and 18 of the 26 (69%) cotrimoxazole-resistant *Klebsiella* strains were resistant to more than 2000 mg/l trimethoprim. These high level trimethoprim-resistant strains invariably carried transferable resistance to at least the sulphonamides, ampicillin and tetracycline, which could be transferred *en bloc* to known sensitive recipients by the process of conjugation. The high incidence of the R-plasmid-mediated resistance to high levels of trimethoprim suggests the presence of a selective pressure from the increased, and probably the uncontrolled, use of trimethoprim/sulphonamide proprietary formulations in the society.

Résumé

La susceptibilité des 40 *Escherichia coli* et 35 espèces de *Klebsiella* isolés d'urine au triméthoprim/sulfamethoxazole (cotrimoxazole) a été essayée. La détermination était sur l'activité des multodisques-cotrimoxazoles normales (25 µg), (Oxoid): sulfafurazole (500 µg) (mastring-S), et la concentration minimum inhibitoire des trimethoprim et sulfamethoxazole appliqué seul aux isolements. Trente deux (80%) des quarante (40) isolements d' *Escherichia coli* et 26 de 35 isolements (74%) des espèces de *Klebsiella* avaient la resistance au cotrimoxazole. Toutes les isolements étaient résistances au plus que 5000 mg/l sulfamethoxazole. Vingt de trente deux (62%) espèces d' *Escherichia coli* resistants au cotrimoxazole et 69% espèces du *Klebsiella* résistants au cotrimoxazole étaient aussi résistants au trimethoprim. Cet augumentation des espèces résistants au trimethoprim invariablement apportait des résistance transféree aux sulfonamides, ampicillin et tetracycline qui peut-etre etait transférée en masse au destinataire sensible par le processus de la conjugation. La fréquence de la résistance obtenue par médiation du R-plasmide aux quantités élevés de trimethoprim propose que on a la présence de poussée selectif de la plus et probablement la utilisation irresponsable des produits déposés du trimethoprim/sulfonamide du monde.

Introduction

Proprietary formulations of trimethoprim, combined with a sulphonamide, are used in many parts of the world as broad spectrum antibacterial agents. In this country the proprietary formulations of trimethoprim/sulphonamide are commonly septrin (Wellcome), bactrim (Roche), nevin (Grunethal-Pharco), lidaprim (Ciba-Geigy) and several others. It is probable that these products are popularly used because of the relative ease of their oral mode of administration.

Surveys of the antibacterial activity of trimethoprim in Great Britain indicated a trend

towards an increasing frequency of resistance to trimethoprim [1–3]. Since 1979, trimethoprim has been used therapeutically in several countries, such as Great Britain, Finland and U.S.A., as a single agent. It has been reported that an increasing proportion of trimethoprim-resistant bacterial isolates in Great Britain were resistant to high levels of trimethoprim (MIC > 1000 mg/l) [4,5] and most of the isolates were also resistant to sulphamethoxazole. It is presently not clear whether the increased trimethoprim resistance is as a result of increased use of trimethoprim rather than an increase of the pool of transmissible genes conferring trimethoprim resistance due to trimethoprim-resistance selection and the consequent change in the genetic location [5,6].

Septrin was the first trimethoprim/sulphamethoxazole proprietary product to be introduced in Nigeria in 1972. Since about 1979 there has been a proliferation of various proprietary formulations of trimethoprim/sulphonamide with concomitant reports of decreasing susceptibility of the local clinical isolates. In a recent investigation, the incidence of urinary tract infection (UTI) with cotrimoxazole-resistant *Escherichia coli* strains among community patients was reported to be about 70% (36 out of 51 patients), compared with 32% (16 out of 49 hospital patients who developed symptoms of UTI while in hospital) in the Lagos area [7].

The frequency and level of trimethoprim/sulphonamide resistance in the Benin City area has not been reported. This study was undertaken to determine the incidence of trimethoprim/sulphonamide resistance among *E. coli* and *Klebsiella* spp. urinary isolates from patients with diagnosed UTI in the Benin City area.

Material and methods

Bacterial strains

Forty *E. coli* and 35 *Klebsiella* spp. isolates from mid-stream urine samples of patients with bacteriologically confirmed UTI ($\geq 10^5$ organisms/ml) were obtained from the Department of Microbiology, University of Benin Teaching Hospital (UBTH) and Diagnostic laboratories in Benin City between April and June 1984. The isolates were further confirmed on receipt by using the standard methods of Cowan and Steel [8]. *Escherichia coli* K12 plasmid free and nalidixic acid-resistant mutant J53-1 (lactose fermenting (Lac^+), proline requiring (pro^-), methionine requiring (met^-); potential recipient of a sex factor (F^-), and nalidixic acid resistant (Nal^r) [9] was used as the R-plasmid transfer recipient from the clinical isolates. *Escherichia coli* NCTC 10481 was employed as the control strain in the antibiotic susceptibility testing.

Media

Nutrient broth No. 2 (Oxoid); diagnostic sensitivity test (DST) agar (Oxoid) containing 4% (v/v) lysed horse blood. MacConkey agar (Oxoid). Davis and Mingioli's minimal salts agar medium was supplemented as required for auxotrophic *E. coli* J53-1 strain [10].

Antibacterial agents

Trimethoprim lactate and sulphamethoxazole were generous gifts from Wellcome (Nigeria Ltd). Nalidixic acid (Sigma Chemical Co.). Multidiscs filter paper (Oxoid) contained nitrofurantoin (NF, 200 μg), tetracycline (Tet. 30 μg), ampicillin (Amp. 25 μg), cotrimoxazole (SXT, 25 μg), nalidixic acid (NA, 30 μg); streptomycin (Sm, 30 μg); gentamicin (CN, 10 μg); sulphafurazole (SF, 500 μg).

Sensitivity test

Antibacterial susceptibility of the isolates was initially determined by the disc-diffusion method using multidiscs of oxoid and mastring-S with inocula of about 10^5 cfu/ml. A reference *E. coli* strain NCTC 10481 was included as control. After incubation at 37°C for 24 h of inhibition around individual disc zones, test and control strains were compared and recorded as sensitive or resistant in relation to the susceptibility of the control strain.

The minimum inhibitory concentrations (MIC) of the antibacterial agents were determined by inoculating the wet-dried DST agar plates containing doubling concentrations of each of trimethoprim and sulphamethoxazole. MICs were recorded as the lowest concentration of the antibacterial agent completely inhibiting visible growth after 48-h incubation at 37°C.

R-plasmid transfer

The method of Jobanputra and Datta [11] was used. Transconjugants were selected on McConkey agar containing ampicillin (20 μg/ml) and nalidixic acid (50 μg/ml). Donors tested for presence of R-plasmids were resistant to trimethoprim/sulphamethoxazole and ampicillin. Transconjugants were repurified on the same selective plates and tested for acquired antibiotic resistance. Confirmation of the phenotypic traits of the transconjugants was by determining the recipients growth requirement (proline and methionine) in minimal salts agar medium.

Curing experiment

The modified method of Rotimi and Duerden [12] using sub-inhibitory concentrations of acridine orange (32 μg/ml) in nutrient broth was used.

Results

Forty and 35 isolates of *E. coli* and *Klebsiella* spp., respectively, were tested; 32 and 26 respectively, were resistant to cotrimoxazole disc (25 μg) (Table 1). The disc-sensitivity tests closely agreed with the MIC tests. Twenty of the *E. coli* and 18 of the *Klebsiella* spp. were resistant to more than 2000 μg/ml trimethoprim. Table 2 shows the cumulative percentage of the sensitivity profile to trimethoprim. Trimethoprim MIC of 2.5 μg/ml was used as the break-point for susceptibility scoring apart from the susceptibility to cotrimoxazole disk. This was based on the MIC of trimethoprim of 1 μg/ml against the control strain *E. coli* NCTC 10481 and the fact that the standard concentration of trimethoprim in the trimethoprim-sensitivity disc is 2.5 μg (Mastring-S). With this classification only 5% (2) of the 40 *E. coli* strains and 8% (3) of the 35 strains of *Klebsiella* spp. fell into the sensitive category in this study. The high level trimethoprim-resistant strains were multiple-resistant to more than three antibiotics. The multiple-resistance, including that towards the sulphonamides, was transferable to sensitive recipient *E. coli* J53-1. Table 3 shows that ampicillin (Amp) and tetracycline resistance (Tet) were invariably present in all the transconjugants, and there were no indications of transposable Tp-resistance since there was no residual resistance trait after curing the R-plasmid.

Isolates of trimethoprim resistance (> 5–640 mg/l) could not transfer trimethoprim resistance, and the sulphonamide resistance in these isolates was not transferable, although they possessed transferable R-plasmids conferring resistance to other antibiotics. The resistance of the transconjugants to the sulphonamides was found to be both R-plasmid and chromosomally mediated because relatively lower levels of transferable sulphonamide resistance were found in most of the transconjugants.

Table 1. Frequency of the *E. coli* and *Klebsiella* spp. urinary isolates resistant to multi-antibiotic discs

Antibiotics tested	*E. coli* $n = 40$	*Klebsiella* spp. $n = 35$
Nitrofurantoin	8	5
Tetracycline	26	27
Ampicillin	34	29
Cotrimoxazole	32	26
Nalidixic acid	0	2
Sulphafurazole	40	35
Streptomycin	16	20
Gentamicin	4	2

Table 2. Cumulative percentage of MIC of trimethoprim (μg/ml) for the bacterial strains

Organism	No.	1	2.5	5	10	20	40	80	160	320	640	1280
E. coli	(40)	1	5	8	—	—	—	10	12	16	18	100
Klebsiella spp.	(35)	—	9	14	—	—	25	—	32	—	40	100

Table 3. Transferable resistance pattern of the *E. coli* and *Klebsiella* spp. urinary isolates

Antimicrobial resistance pattern transferred	No. donor isolates that transferred plasmid	
	E. coli	*Klebsiella* spp.
Tp	0	0
Su	0	0
Tp Su	0	0
Tp Su Tet Amp	20	18
Tp Su Tet Amp Sm	20	18
Tp Su Tet Amp Sm CN	3	1
Tp Su Tet Amp Sm CN NT	2	2

Tp: trimethoprim; Su: sulphafurazole or sulphamethoxazole; Tet: tetracycline; Sm: streptomycin; Amp: ampicillin; CN: gentamicin; NT: nitrofurantoin.

Discussion

The susceptibility of urinary bacterial isolates from UTI cases to trimethoprim and cotrimoxazole has been studied in many countries, notably in Great Britain and Finland [5,6, 13–16]. The results of this study showed a high incidence of high level resistance of *E. coli* and *Klebsiella* spp. to trimethoprim, as a result of the continuous selection of transferable Tp-resistance gene pool in the resistant strains. In Nigeria, it is commonly known that despite all efforts to officially control antibiotic usage, antibiotics can still be freely purchased in open markets while the extent and level of trimethoprim resistance has not actually been known. A previous report showed that in the Lagos area there was a high incidence of *E. coli* resistance to cotrimoxazole [7]. This study agrees with that report and also showed that the prevalence of trimethoprim-resistant *E. coli* and *Klebsiella* spp. was high and R-plasmid mediated. The high level of transferable R-plasmid in the *Klebsiella* spp. isolates in this study, however, did not agree with the earlier reports that *Klebsiella* spp. had a little tendency to confer R-plasmid-mediated trimethoprim-resistance [6,17]. This indicates the need for regular monitoring of the susceptibility of local isolates to antibiotics, in order to understand the prevailing antibiotic activity against the local bacterial strains. The results of this study also indicate that the high incidence of transferable trimethoprim was probably due to the uncontrolled use of trimethoprim/sulphonamide formulations, as well as the increased selection of the trimethoprim/sulphonamide gene pool. This is because there was both chromosomal- and R-plasmid-mediated resistance. However, there is no indication of transposable Tp-resistance in this study since all the cured transconjugants do not retain any residual trimethoprim resistance. No attempt has been made to report the nature of the R-plasmids conferring trimethoprim/sulphamethoxazole resistance in this study. Further studies will be concerned with the various plasmids isolated in this study, with the aim of characterizing the plasmids and to gain an understanding of their inter-relationships and routes of transmission throughout the community.

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