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Resistance of *Klebsiella* Species Isolates From Two Institutions in Nairobi, Kenya, to Commonly Prescribed Antimicrobial Agents

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***Klebsiella* species isolates collected from the Kenyatta National Hospital and the Centre for Microbiology Research of the Kenya Medical Research Institute in Nairobi, Kenya were identified and screened for resistance to commonly prescribed antimicrobial agents. Most of the isolates were found to exhibit resistance to more than three agents amongst those tested by both disk diffusion tests and minimum inhibitory concentration determination.**

Key words: *Klebsiella*, resistance, minimum inhibitory concentration, disk diffusion

INTRODUCTION

The *Klebsiella* species have been shown to be important opportunistic pathogens featuring prominently among causative agents of nosocomial infections [1] and community acquired bacteraemia as well as infections at other sites [2]. Epidemics caused by antimicrobial resistant *Klebsiella* species have led to closures of hospital specialist units or even whole hospitals [3]. At the Kenyatta National Hospital, the major public referral and teaching hospital in Kenya, high prevalence of infection with *Klebsiella* species has been reported [4-5]. The current study was undertaken to find out the prevalence of resistance to antibimicrobial agents used in the treatment of urinary and non-urinary isolates collected at the Kenyatta National Hospital and at the Centre for Microbiology Research (CMR) of the Kenya Medical Research Institute.

EXPERIMENTAL

Samples

Samples of urine, blood, cerebrospinal fluid, sputum, stool and pus as well as vaginal and throat swabs were examined for the presence of *Klebsiella* species at the routine Microbiology Laboratory of the Department of Medical Microbiology of the University of Nairobi (based at the Kenyatta National Hospital) and at the CMR of the Kenya Medical Research Institute between October 1991 and March 1992. A total of 118 isolates were obtained from samples from the Kenyatta National Hospital and the CMR. An additional 16 isolates that had already been identified were obtained from the Kenya Medical Research Institute/Wellcome Trust Research Laboratory (KEMRI/WTRL), Nairobi. The isolates consisted of 86 urinary and 48 non-urinary isolates. These were subjected to disk diffusion tests at the Kenyatta National Hospital and the CMR laboratories and minimum inhibitory concentration (MIC) determination at the KEMRI/WTRL.

Disk diffusion tests

The tests were carried out with a bacterial cell suspension adjusted to a turbidity comparable to that of a 0.5 McFarland turbidity standard (approximately 10^8 colony forming units per ml).Urinary isolates were tested with disks containing predetermined amounts of ampicillin (10 µg), co-trimoxazole (1.25/23.75 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), sulphamethoxazole (300 µg), streptomycin (10 µg) and tetracycline (30

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µg). Non-urinary isolates were tested with disks containing ampicillin (10 µg), chloramphenicol (30 µg), co-trimoxazole (1.25/23.75 µg), gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), sulphamethoxazole (300 µg) and tetracycline (30 µg). Any isolate that appeared resistant to four or more antimicrobial agents was tested with single disks containing amikacin (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), or enoxacin (10 µg).

Determination of Minimum Inhibitory Concentrations

Eighty six isolates picked at random from among the 134 originally collected were used for the determination of the MIC by the agar dilution method on Drug Sensitivity Testing (DST) agar (Oxoid Ltd, Basingstoke, Hampshire, England). The MICs of amoxycillin, co-amoxiclav, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, sulphamethoxazole, tetracycline, trimethoprim, and co-trimoxazole against the 86 isolates were determined. *Escherichia coli* strain ATCC 25922 obtained from the National Public Health Laboratories, Nairobi, Kenya was used as the reference strain for all MIC determinations.

RESULTS

Identification and distribution of *Klebsiella* isolates from various specimens and swabs

The 134 isolates were identified as *K. pneumoniae* subspecies *pneumoniae*, and *ozaenae or K. oxytoca*. One hundred and seventeen isolates (87.3 %) were *K. pneumoniae* subspecies *pneumoniae*, while fifteen isolates (11.2 %) were *K. oxytoca* and two isolates (1.5 %) were *K. pneumoniae* subspecies *ozaenae*.

Disk diffusion tests

Table 1 shows the resistance levels of the *Klebsiella* isolates to each of the drugs used in the disk diffusion test. It was found that all 86 urinary isolates (100 %) were resistant to ampicillin. Resistance to the other drugs among urinary isolates was found to rise in the order of nalidixic acid (3.5 %), nitrofurantoin (9.3 %), gentamicin (66.0 %), tetracycline (77.9 %), streptomycin (90.7 %), co-trimoxazole (96.5 %), and sulphamethoxazole (97.7 %).

Among the non-urinary isolates, resistance was observed in the increasing order of kanamycin (47.9 %), gentamicin (52.1 %), chloramphenicol (66.7 %), streptomycin and tetracycline (68.8 %), co-trimoxazole (72.9 %), sulphamethoxazole (85.4 %) and ampicillin (97.9 %). The isolate that was susceptible to ampicillin was one of the two *Klebsiella pneumoniae* subspecies *ozaenae* isolates encountered in this study. Besides the results presented in Table 1, two isolates were found to be resistant to ceftriaxone, but not to amikacin, aztreonam, cefotaxime, ceftazidime, cefuroxime or enoxacin.

Table 2 shows a comparison of the susceptibilities of urinary and non-urinary isolates to the various antimicrobial agents used. Similar antimicrobial agents were used for both groups of isolates except that nitrofurantoin and nalidixic acid were included only for urinary isolates while chloramphenicol and kanamycin were tested on non-urinary isolates only.

For both the urinary and non-urinary categories, it was observed that most of the isolates were resistant to ampicillin, sulphamethoxazole and co-trimoxazole. Additionally, there was no significant difference in resistance prevalence between the urinary and non-urinary isolates.

Table 1: Number of isolates resistant to each antimicrobial agent as determined by disk diffusion sensitivity tests.

Antimicrobial agent	Number resistant urinary isolates (%)	Number resistant non-urinary isolates (%)
Ampicillin	86 (100)	47(97.9)
Sulphamethoxazole	84 (97.7)	41(85.4)
Co-trimoxazole	83 (96.5)	35(72.9)
Streptomycin	78 (90.7)	35(72.9)
Tetracycline	67 (77.9)	33(68.8)
Chloramphenicol	-	32(66.7)
Gentamicin	57 (66.3)	25(52.1)
Kanamycin	-	23(47.9)
Nitrofurantoin	8 (9.3)	-
Nalidixic acid	3 (3.5)	-

- = Not tested in these isolates

Table 2: Comparison of prevalence of resistance to various antimicrobial agents tested against urinary and non-urinary *Klebsiella* isolates

Antimicrobial agent	Percentage isolates		χ^2 Yate's Corrected	p-value
	Urinary	Non-urinary		
Ampicillin	100	97.9	0.01	0.9199
Gentamicin	66.3	52.1	2.86	0.0906
Streptomycin	90.7	72.9	3.52	0.0605
Sulphamethoxazole	97.7	85.4	1.57	0.2097
Co-trimoxazole	96.5	72.9	6.22	0.0126
Tetracycline	77.9	68.8	0.87	0.3507

$p \leq 0.01$ is significant.

Table 3: The MIC_{50}, MIC_{90} and MIC range of antimicrobial agents against 86 *Klebsiella* isolates and the resistance prevalence to each agent.

Antimicrobial Agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	Number of resistant isolates (%)
Amoxycillin	128	128	1 - 128	85 (98.8)
Sulphamethoxazole	>1024	>1024	16 - >1024	76 (88.4)
Co-trimoxazole	>32/640	>32/640	0.25/5 - >32/640	67 (77.9)
Trimethoprim	128	>256	0.25 - >256	67 (77.9)
Tetracycline	64	128	2 - 128	65 (75.6)
Chloramphenicol	64	128	1 - 128	62 (72.1)
Gentamicin	32	128	1 - 128	53 (61.6)
Co-amoxiclav	32/16	32/16	1/0.5 - 64/32	46 (53.5)
Ciprofloxacin	0.06	0.25	0.03 - 1	0 (0.0)

Minimum inhibitory concentrations.

The minimum inhibitory concentration (MIC) of 9 antimicrobial agents was determined against 86 *Klebsiella* isolates picked at random from among both urinary and non-urinary isolates. The MIC_{50} and MIC_{90} were considered an indication of the overall response of the 86 isolates to each antimicrobial agent (Table 3). The values of both MIC_{50} and MIC_{90} for all the agents tested except ciprofloxacin were above the MIC break points for resistance as defined by the National Committee for Clinical Laboratory Standards [6].

From the results shown in Table 3 it is clear that the smallest number of resistant isolates was observed with co-amoxiclav (53.5 %) while the highest was observed with amoxycillin (98.8 %). It was observed that resistance to trimethoprim and to co-trimoxazole appeared in a similar number of isolates (77.9 %) while resistance to sulphamethoxazole alone appeared in a higher number of isolates (88.4 %). Further, all the isolates susceptible to trimethoprim were also found to be susceptible to co-trimoxazole.

DISCUSSION

The prevalence of strains in this study reflects a trend in which K*lebsiella pneumoniae* subspecies *pneumoniae* is reported to be responsible for more infections than *Klebsiella oxytoca* and the other subspecies put together [7].

Disk diffusion sensitivity tests showed that prevalence of resistance was above 66 % for all the drugs tested except for nalidixic acid and nitrofurantoin among the urinary isolates and for gentamicin and kanamycin among the non-urinary isolates. All except 8 among the non-urinary isolates were resistant to at least three drugs. The same trend was observed when the minimum inhibitory concentrations were determined.

All drugs were tested in concentrations up to at least three MIC levels above the National Committee for Clinical Laboratory Standards defined break points for resistance [6]. The MIC_{50} and MIC_{90} of all the drugs tested except that of ciprofloxacin were at least two levels above the NCCLS break points. These results show that most of the *Klebsiella* isolates dealt with in this study exhibited very high levels of resistance to the antimicrobial agents tested. Moreover, they exhibited multiple resistance to the agents. The isolates were, however, susceptible to amikacin, aztreonam, enoxacin, and the third generation cephalosporins. Only two isolates were confirmed to have been resistant to ceftriaxone. The presence of those two isolates resistant to ceftriaxone raises the possibility of the presence of extended spectrum beta-lactamases among the isolates. An extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* among neonatal pathogens has been reported previously [8]. It has also been reported that resistance to third generation cephalosporins may exist and yet not be shown by routine disk sensitivity tests unless disks containing clavulanic acid are included to reveal such resistance during routine testing [9]. It would thus be important to include these disks in all tests involving cephalosporins as a surveillance strategy against development of extended spectrum of beta-lactamases.

The very high levels of resistance to gentamicin, chlorampenicol, co-timoxazole, trimethoprim and sulphamethoxazole show that these agents have a limited capacity to treat infections caused by *Klebsiella* species. It has been reported that nitrofurantoin does not share cross-resistance with commonly prescribed antimicrobial agents. It can be used for the empirical eradication of uropathogens [25].

All isolates were susceptible to ciprofloxacin, a fluoroquinolone. Unlike the first generation quinolones represented by nalidixic acid, fluoroquinolone are reported to have a lower propensity for the development of resistance and to have superior activity against uropathogens in comparison to co-trimoxazole [10-11]. Moreover, because of the continued development of compounds with improved antimicrobial spectra and pharmacokinetics, the fluoroquinolones could become the major antibacterial agents of the 21st century. However, they should be used in an educated

fashion, based on a careful balance between their ease of use versus the risk of emerging resistance and toxicity [11-12].

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