

Multicenter Evaluation of Resistance Patterns of *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* spp and *Shigella* spp isolated from Clinical Specimens in Brazil: RESISTNET Surveillance Program

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Surveillance programs are essential to detect the increase of antimicrobial resistance, and several different programs are being conducted in many countries. The RESISTNET is a surveillance program for bacterial resistance against several antimicrobial agents initiated in 1998 among Latin American countries. In Brazil, several centers were invited to join this surveillance and a total of 11 centers (6 from São Paulo and 5 from other states) participated in the study. All results were analyzed using the WHONET program. A total of 894 *Escherichia coli*, 386 *Klebsiella pneumoniae*, 70 *Shigella* spp and 57 *Salmonella* spp strains were analyzed in this study from April, 1998, to April, 1999. Susceptibility testing was performed by the disk diffusion method using NCCLS 1998 guidelines for several different drugs. For all strains, imipenem was the most effective drug (100% of the strains were susceptible). *Klebsiella pneumoniae* presented a high resistance rate to ampicillin (96.4%). The rate of probable ESBL producers among *K. pneumoniae* strains was 36.3%, most of them being isolated from catheters (58.8%). Among all *Escherichia coli* strains analyzed, the highest resistance rate was found for trimethoprim/sulfamethoxazole (46.9%) and the majority of the resistant strains were isolated from urine samples (47.8%). Among *Salmonella* spp, the resistance rates were low for all antibiotics tested. For *Shigella* spp strains there was a high resistance to trimethoprim/sulfamethoxazole (80.0%). No resistance to ceftriaxone was observed in these strains. Surveillance of antimicrobial resistance is critical for the successful management of infectious diseases. The results of this survey show significant resistance rates among these bacteria which are responsible for several types of human infections.

Key words: Enterobacteria, antimicrobial resistance, surveillance programs, WHONET.

The increase in antimicrobial resistance of bacteria to different antimicrobials leads many countries to initiate surveillance programs [1-3]. From all bacteria that have developed resistance, the Enterobacteriaceae are a group of special concern

Received on 25 August 2000; 14 November 2000.

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The Brazilian Journal of Infectious Diseases 2001;5(1):8-12
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1413-8670

due to the high increase of resistance recently observed. *Salmonella* spp and *Shigella* spp are clear examples, with an increase in resistance to antibiotics that are considered drugs of choice in the treatment of infections caused by these bacteria. Some strains of *Klebsiella pneumoniae* and *Escherichia coli* produce different enzymes (ESBL, carbapenases, beta-lactamases) that are changing the therapeutic usefulness of some antimicrobials.

There are some situations that physicians and microbiologists must know in order to have a better understanding of the dissemination and control of bacterial resistance. Surveillance programs are essential

to meet this goal. In 1998, the RESISTNET program was initiated to analyze the resistance patterns of several bacteria from Latin American countries.

This study presents data pertaining to antimicrobial resistance of *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella* spp, and *Salmonella* spp collected from 11 centers around Brazil. All data was entered into the WHONET program [4]. The WHONET program is a computer program designed to monitor and manage information about bacterial resistance by merging from many centers and providing an overview of global antimicrobial resistance patterns.

Materials and Methods

In this study, resistance data were collected from April, 1998, to April, 1999, from 11 centers in Brazil. Six centers were located in the city of São Paulo (Santa Casa de Misericórdia de São Paulo, Hospital São Paulo, Laboratório de Investigação Médica - LIM-54- Divisão de Moléstias Infecciosas e Parasitárias do HC FMUSP, Hospital Emilio Ribas, Faculdade de Ciências Farmacêuticas USP and Hospital Heliópolis). The other five centers were from different cities around the country (Brasília – Laboratório Exame; Curitiba – Hospital das Clínicas de Curitiba; Salvador - Hospital Aliança; Fortaleza – Laboratório Louis Pasteur; Porto Alegre – Hospital das Clínicas de Porto Alegre).

A total of 894 *Escherichia coli*, 386 *Klebsiella pneumoniae*, 70 *Shigella* spp, and 57 *Salmonella* spp strains from inpatients and outpatients from varied anatomical sites were analyzed in this study. Susceptibility tests were performed by the disk diffusion method and the results were evaluated according to the NCCLS 1998 guidelines [5]. The following drugs were tested against *E.coli* and *K. pneumoniae*: ampicillin, cephalotin, ceftriaxone, ciprofloxacin, gentamicin, imipenem, ampicillin/sulbactam, and trimethoprim/sulfamethoxazole. For *Salmonella* spp and *Shigella* spp, the following drugs were tested: ampicillin, chloramphenicol, ceftriaxone, ciprofloxacin and trimethoprim/sulfamethoxazole. All results were entered into the WHONET program (WHONET4).

Quality control was performed with the following ATCC strains: *Staphylococcus aureus* 25923, *E. coli* 25922, and *P. aeruginosa* 27853.

Results and Discussion

The resistance rates for all 4 bacteria analyzed in this study are presented in Figure 1. Table 1 shows the difference in the resistance rates for these bacteria isolated from outpatients and inpatients.

Antimicrobial Susceptibility of *Klebsiella pneumoniae*

From the 386 strains of *K. pneumoniae* analyzed, 270 (70%) were from inpatients and 116 (30%) were from outpatients. Among all *K. pneumoniae* analyzed, 31.9% (123) were isolated from urine, 13.7% (53) from blood, 9.3 % (36) were isolated from samples of the lower respiratory tract, 5.7% (22) from abscesses, 4.1% (17) from catheters and 35.4% were isolated from several other clinical specimens.

Imipenem was the most efficient drug against *K. pneumoniae* (all strains were susceptible). The percentage of resistance to ciprofloxacin was 2.2% and resistance to trimethoprim/sulfamethoxazole (TSX) was 33.3%. The percentage of resistance among outpatients to ampicillin/sulbactam was 11.3% and, to ampicillin alone, the resistance was 94.5%. A similar profile was observed for inpatients, where we had 33.7% resistance to ampicillin/sulbactam, and 97% to ampicillin alone. There was 3.9% resistance to ceftiofloxacin, probably the same percentage of ampC *K. pneumoniae* strains. The highest percentage of *K. pneumoniae* strains producing ampC (5.9%) were found in strains isolated from catheters. Among all strains tested, we found 36.3% of strains to be possible ESBL producers when NCCLS criteria (inhibition zones ≤ 25 mm) were used to evaluate ceftiofloxacin activity. From the 53 strains of *K. pneumoniae* isolated from blood samples, 47.2% were probable ESBL producers and over half (58.8%) of those isolated from catheters were also probable ESBL producers. In all strains isolated from urine samples, 24.4% were probable ESBL producers. From

the 123 strains isolated from urine samples, 41 (33%) were from outpatients and 17.1% of these strains were found to be probable ESBL producers. These data indicate that this is an important point to be considered when treating this kind of infection. The high rates of ESBL producing strains in Latin American countries have been described in several other studies [2, 6, 7]. The rates found in these surveillance studies show similar percentages among *K. pneumoniae* strains. In a multicenter study from 5 European countries, the percentage of probable ESBL producers was lower than the percentage found in our study. In that study the highest percentages were found in Portugal (36.0%) and in France (26.0%) [1]. Another interesting observation in this study was the important difference in the resistance rates for aminoglycosides between strains isolated from inpatients and outpatients (Table 1). This could be due to the extensive use of this class of antimicrobials in the hospital environment.

Antimicrobial Susceptibility of *Escherichia coli*

From the 894 strains of *E. coli* analyzed 67.1% (600) were isolated from inpatients and 32.9% (294) from outpatients (Table 1). Of all the strains of *E. coli* analyzed, 52.7% (471) were isolated from urine samples, 7.6% (68) from abscess, 6.4% (57) from blood, 2.8% (25) from surgical wounds, 0.2% (2) from catheters, and 30.3% (271) were isolated from several other clinical specimens. For all strains, there was a 55.0% resistance rate to ampicillin; however, the association with sulbactam showed a significantly lower resistance rate (12.0%).

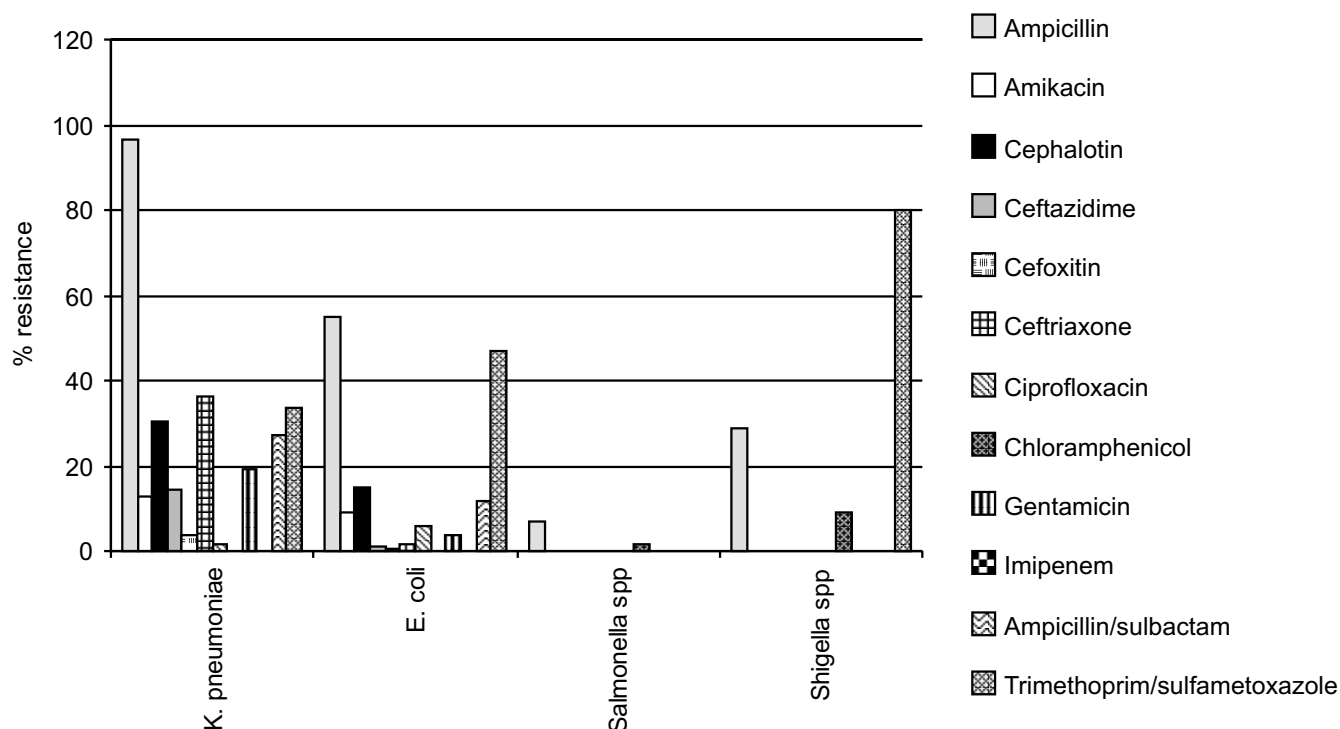
A similar profile was observed when we analyzed strains from inpatients and outpatients. Among inpatients, all strains showed 13.7% resistance to ampicillin/sulbactam and 55.9% resistance to ampicillin alone. There was a 46.9% resistance to TSX among all strains (Figure 1). A higher percentage of strains resistant to TSX was observed in strains isolated from urine samples (47.8%). Cephalotin is another drug commonly used for the treatment of urinary infections in Brazil, and we observed 11.5% resistance and 32.5% intermediate resistance to this drug. This may

be due to the extensive use of cephalotin for treating this type of infection. The scenario was similar for strains isolated from blood cultures, with all strains presenting a high percentage of resistance to cephalotin (23.6%) and to TSX (41.8%).

For all of the *E. coli* strains analyzed, there was a 5.9% resistance rate to ciprofloxacin. In strains isolated from urine, the percentage increased to 6.4% and, for strains isolated from blood cultures, the resistance rate was 5.5%. Based on the same criteria used to analyze *K. pneumoniae* ESBL producer strains, there was a lower percentage of *E. coli* strains probable ESBL producers (8.0%). This percentage is lower than the one presented by Gales, et al. [6] but very similar to the rate found by Hsiung, et al. [7]. There was no important difference between the resistance rates of *E. coli* strains isolated from inpatients and outpatients (Table 1).

Antimicrobial Susceptibility of *Salmonella* spp and *Shigella* spp

Of all *Salmonella* spp strains analyzed, 66.7% (38) were from inpatients and 33.3% (19) from outpatients. For *Shigella* spp strains, 81.4% (57) were from inpatients and 18.6% (13) from outpatients. Of the 57 strains of *Salmonella* spp analyzed, 35.0% were isolated from blood, 49.1% from stool, and 15.9% from other sites (urine, abscess, bile). Of the 70 strains of *Shigella* spp, 20.0% were isolated from blood, 74.3% from stool, and 5.7% from other sites. *Shigella sonnei* was the most frequent species isolated (63.0%). All *Salmonella* species were susceptible to TSX, ceftriaxone and ciprofloxacin (Figure 1) (Table 1). The rate of resistance to ampicillin (7.0%) is rather low when compared to the resistance rates found in other Latin American countries (Argentina 60.3%, Colombia 20.0%, Mexico 16.7%) [8]. If we analyze the resistance patterns of the isolates obtained from blood and stool to ampicillin, we note that there is no resistance among strains isolated from blood but among strains isolated from stool, 10.7% are resistant. This might be because some of these infections are treated with ampicillin and other common drugs that are very popular in

Figure 1. Antimicrobial resistance rates among all strains analyzed (1,407 strains)**Table 1:** Percentage of resistance of *K. pneumoniae*, *E. coli*, *Salmonella* spp and *Shigella* spp from inpatients and outpatients

Antimicrobial	<i>K. pneumoniae</i>		<i>E. coli</i>		<i>Salmonella</i> spp		<i>Shigella</i> spp	
	In (n=270)	Out (n=116)	In (n=600)	Out (n=294)	In (n=38)	Out (n=19)	In (n=57)	Out (n=13)
Ampicillin	97.0**	94.5	55.9	53.1	5.3	10.5	33.3	7.7
Amikacin	16.3	4.3	1.2	0.3	-	-	-	-
Cephalotin	38.3	12.9	15.4	13.7	-	-	-	-
Ceftazidime	18.5	4.3	0.8	1.4	-	-	-	-
Cefoxitin	4.8	1.7	0.3	0.7	-	-	-	-
Ceftriaxone*	42.2	22.4	9.0	5.8	0	0	0	0
Chloramphenicol	-	-	-	-	0	5.6	10.9	0
Ciprofloxacin	2.2	0	5.0	7.9	0	0	0	0
Gentamicin	25.2	5.2	4.4	3.1	-	-	-	-
Imipenem	0	0	0	0	-	-	-	-
Ampicillin/ Sulbactam	33.7	11.3	13.7	8.6	-	-	-	-
Trimethoprim Sulfamethoxazole	39.3	20.7	47.4	45.7	0	0	82.5	69.2

In: inpatients; Out: outpatients; -: not tested. *: considering ceftriaxone ≤ 25 mm; **: percentage of resistance.

some countries. Chloramphenicol is another antimicrobial that presented different resistance rates in blood and stool isolates, with 0% and 4% resistance, respectively.

There was a high resistance rate to TSX among *Shigella* species (80.0%), with similar resistance in strains isolated from blood (76.9%) and stool (83.0%). All strains showed 28.6% resistance to ampicillin. *Shigella* spp isolated from stool samples showed 30.2% resistance to ampicillin, and those isolated from blood showed 23.1% resistance. The resistance rates to ampicillin are higher in other Latin American countries than the ones we found in this study [8]. The same was observed with chloramphenicol where we observed a 9.0% resistance, while the resistance rates were much higher in other countries (over 40.0%). No resistance was observed to ceftriaxone.

The results of this study show the importance of these 4 bacteria as causes of infections. Furthermore, we demonstrated that important antimicrobial resistance rates exist among the 4 isolates analyzed. In making therapeutic choices, it is very important to know the antimicrobial resistance rates of these bacteria when selecting empiric therapy for a patient. Surveillance studies play a very important role for the management of infectious diseases. It is important to conduct these studies to obtain data which allow us to recognize the susceptibility patterns of different bacteria species.

Acknowledgements

The RESISTNET-Group: H. S. Sader (São Paulo Hospital); C. Mendes (LIM-54, Infectious Diseases Department, HC-FMUSP, São Paulo); I. Mimiça (Santa Casa da Misericórdia de São Paulo); Flávia Rossi (Emilio Ribas Hospital, São Paulo); E. Mamizuca (Faculty of Science Pharmaceutical, USP, São Paulo); A. Segura (Exame Laboratory, Brasília); L. Della Costa (Clinical Hospital, Curitiba); José Francisco (Aliança Hospital, Salvador); T. Bandeira (Louis Pasteur Laboratory, Fortaleza); A. Barth (Clinical Hospital, Porto Alegre).

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