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INHIBITORY EFFECT OF DIFFERENT ANTIBIOTICS ON NOSOCOMIAL PATHOGEN *SERRATIA MARCESCENS*

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ABSTRACT

The *S. marcescens* has recognized as causes of many hospital epidemics and a causative agent of hospitalized nosocomial infection. It causes secondary infections such as urinary, respiratory, wound and septic arthritis, peritonitis and sinusitis. *S. marcescens* constitutively possesses chromosomally encoded, inducible Amp^c β-lactamases and may acquire plasmid- mediated extended-spectrum β-lactamases (ESBLs). They have ability to develop resistance to many β-lactame antiobiotics. In this studies totally 222 *S. marcescens* isolate were used for testing antibiotic sensitivity against antibiotic like Ampicillin, Gentamicin, Cefotaxime, Chloramphenicol, Amikacin, Aztreonam, Ceftazidime, Cephalothin, and Ciprofloxacin. The antibiotic sensitivity were analysed in the presence of zone of inhibition around the antibiotic disc. All 222 strains of *S. marcescens* gave maximum susceptible (13mm) to ciprofloxacin antibiotic and also *S. marcescens* gave different resistant spectrum to the other 8 antibiotics. Ciprofloxacin is an effective antibiotic for *S. marcescens* infections.

Keywords: *S. marcescens*, nosocomial infection, septic arthritis, peritonitis, sinusitis.

INTRODUCTION

Introduction of antimicrobials in the therapeutics of infectious diseases was described over 2,500 years ago. At that time, they were regarded as the solution to all diseases caused by microorganisms (Riberro Filho and Fernander, 2000). *S. marcescens* has been recognized as the cause of many hospital epidemics (Farmer *et al.*, 1976 and Wong *et al.*, 1999) and a causative agent of hospitalized nosocomial infection (Yu, 1979). It causes several diseases as a secondary infection such as urinary, respiratory, wound and septic arthritis, peritonitis and sinusitis (Eisenstein *et al.*, 2000). *S. marcescens* constitutively possesses chromosomally encoded, inducible Amp^c β-lactamases and may acquire plasmid- mediated extended-spectrum β-lactamases (ESBLs). Therefore, they have ability to develop resistance to many β-lactame antiobiotics (Bennett and Chopra, 1993).The antibiotic resistance is widespread and indiscriminate use has the selection of resistant strains and their empirical use, the lack of standardization for therapeutic prescription, among other factors, have led to a selective pressure on microorganisms, leading to difficult to treat, multi-resistant strains (Garner *et al.*, 1998).However, their widespread, indiscriminate and empirical uses of antibiotics have caused the selection of resistant strains and have led to a selective pressure on microorganisms, leading to difficult to treat multiresistant strains. This has created impasses in the treatment of patients in hospitals (Riberro Filho and Fernander, 2000). The present study deals with the antimicrobial sensitivity

profile of *S. marcescens* to find the effective antimicrobial agent for the treatment of *S. marcescens* infections.

MATERIAL AND METHODS

Seed culture preparation: Totally 222 *S. marcescens* isolates (105 *S. marcescens* isolates of patient from clinical samples, 58 isolates of *S. marcescens* from environmental samples and 59 isolates from cockroaches) were used for antimicrobial sensitivity tests using the disc diffusion method, according to the National Committee for Clinical Laboratory standards (NCCLS) recommendations for the determination of antimicrobial susceptibility (NCCLS, 2004). All 222 strains were seeded in agar slants and incubated at 37ºC for 24 hours. The bacterial inoculates were prepared in 0.8% sterile saline solution by inoculating a loop of culture in 0.5ml of sterile saline solution.

Antibiotic assay: From the broth inoculate preparation (0.5 ml of 0.8% sterile saline solution) 0.1 ml of culture was taken and seeded in Muller Hinton agar, the antibiotic disc was distributed in an equidistant fashion and the plates were incubated at a temperature of 37ºC for 24 hours by following the method of Bauer *et al.* (1966). The antimicrobial agents used were ampicillin (10 µg), gentamicin (10 µg), cefotaxime (30 µg), chloramphenicol (30 µg), amikacin (30 µg), aztreonam (30 µg), ceftazidime (30 µg), cephalothin (30 µg) and ciprofloxacin (5 µg). The susceptible and resistance isolates were identified in the presence of zone of inhibition around the antibiotic discs.

RESULTS AND DISCUSSION

Clinical specimen isolates: 105 isolates of *S. Marcescens* obtained from patient's clinical specimens were tested for antimicrobial susceptibility. Table 1 shows the frequency of clinical specimen isolates of *S. marcescens* in relation to antimicrobial susceptibility. 1 mm zone of inhibition was found to ampicillin, 3 mm to gentamicin, 7 to cefotaxime, 6 mm to chloramphenicol, 4 mm to amikacin, 3 mm to aztreonam, 5 mm to ceftazidime, 3 mm to caphalothin, and 13 mm to ciprofloxacin.

Environmental and HCW specimen isolates: Totally 58 *S. marcescens* isolates of environmental source were submitted for antimicrobial susceptibility. Table 2 shows the frequency of environmental isolates of *S. marcescens* in relation to antimicrobial susceptibility. 1 mm zone of inhibition was found to ampicillin, 3 mm to gentamicin, 7 to cefotaxime, 6 mm to chloramphenicol, 4 mm to amikacin, 3 mm to aztreonam, 5 mm to ceftazidime, 3 mm to caphalothin, and 13 mm to ciprofloxacin.

Cockroaches' isolates: 59 isolates of *S. marcescens* from cockroaches in hospital environments were tested for its antimicrobial susceptibility. Table 3 shows the frequency of antimicrobial susceptibility of *S. marcescens.* 1 mm zone of inhibition was found to ampicillin, 3 mm to gentamicin, 7 to cefotaxime, 6 mm to chloramphenicol, 4 mm to amikacin, 3 mm to aztreonam, 5 mm to ceftazidime, 3 mm to caphalothin, and 13 mm to ciprofloxacin.

Table 1. Clinical specimen isolates of *S. marcescens* antimicrobial sensitivity.

Total no. of isolates	Antibiotic	Zone of inhibition (in mm)
105	Am	1
105	Gn	3
105	Ctx	7
105	Chl	6
105	An	4
105	Azt	3
105	Caz	5
105	Cap	3
105	Cip	13

Table 2. Environmental & HCW specimen isolates of *S. marcescens* antimicrobial sensitivity.

Total no. of isolates	Antibiotic	Zone of inhibition (in mm)
58	Am	1
58	Gn	3
58	Ctx	7
58	Chl	6
58	An	4
58	Azt	3
58	Caz	5
58	Cap	3
58	Cip	13

Table 3. Cockroaches specimen isolate of *S. marcescens* antimicrobial sensitivity.

Total no. of isolates	Antibiotic	Zone of inhibition (in mm)
59	Am	1
59	Gn	3
59	Ctx	7
59	Chl	6
59	An	4
59	Azt	3
59	Caz	5
59	Cap	3
59	Cip	13

Figure 1 shows the antibiotic sensitivity profile of *S. marcescens* to different antibiotic drug. The data suggest that all 222 isolates of *S. marcescens* were showed maximum zone of inhibition only to ciprofloxacin. The sensitivity of analyzed *S. Marcescens* isolates to the antibiotics was given in Figure 2.

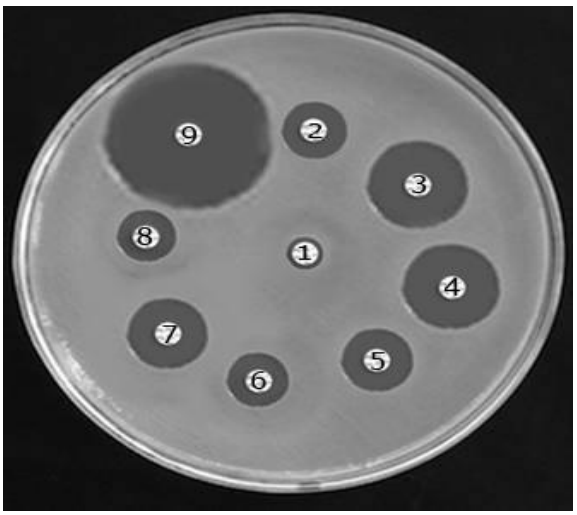

Figure 1. Shows antibiotic zone of inhibition of *S. marcescens* isolates on Muller Hinton agar. 1-Ampicillin,**2 G**entamicin,**3-**Cefotaxime,**4-C**hloramphenicol,**5-**Amikacin,**6-A**ztreonam,**7-C**eftazidime,**8-C**aphalothin,**9-** Ciprofloxacin.

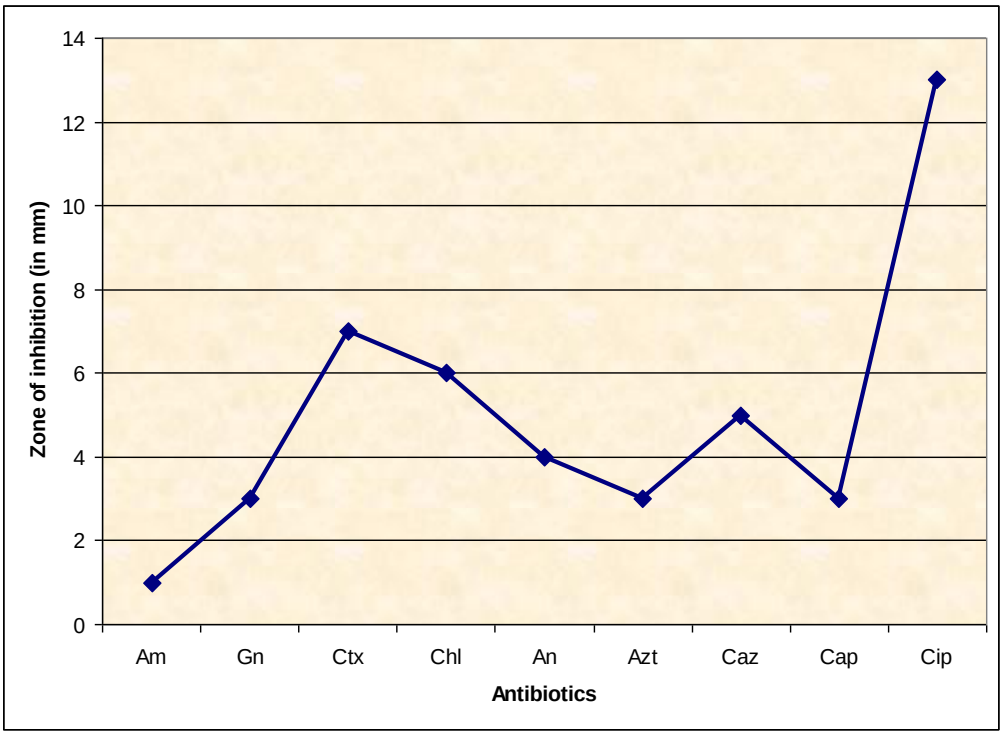

Figure 2. Resistance spectrum of *S. Marcescens* to antimicrobials (Am-Ampicillin, Gn-Gentamicin, Ctx-Cefotaxime, Chl-Chloramphenicol, An-Amikacin,Azt-Aztreonam,Caz-Ceftazidime,Cap-Caphalothin,Cip- Ciprofloxacin).

The majority of strains proved resistance to many antibiotics. Among this study *S. marcescens* strains, there was a large percentage of strains which were resistant to ampicillin and also reported by many authors (Lohr *et al.*, 1994). Ampicillin is clinically useful due to the inhibition of β-lactamase, which is effective in the treatment of serious infection in the respiratory tract (Isenberg, 1992), urinary (Livemore, 1995), gynecological and septicemia triggered by β-lactamase producing organisms (Haddy *et al.*, 1996). The results of study confirm the resistance rates of *S. marcescens* were submitted to ampicillin is 1 mm zone of inhibition to 105 clinical specimens isolate, 58 environmental and HCW specimens isolate, and 59 of cockroach specimens isolates. The overall resistance rate of *S. marcescens* to ampicillin was 89.1 % (198/222).

Gentamicin is a broad spectrum antibiotic that acts against the both Gram-positive bacteria and Gram-negative bacteria. It's mainly active against Gram-negative particularly enterobacteria (Livemore, 1995). However in this study *S. marcescens* isolates of clinical specimen 57.1 % (60/105), environments and HCW sources 74.1% (43/58), and cockroaches sources 81.3% (48/59) were showed resistance to gentamicin. Many other authors also reports about the phenomenon of resistance to amino glycoside antibiotics gentamicin (Zhang, 1991). The enterobacteria isolates from the cockroaches were relatively resistant to gentamicin; 21.5% for *K. pneumoniae*, 14% for *E. aerogenes*, 13% for *S. marcescens* (Jarvis and Martone, 1992). The overall resistance rate of *S. marcescens* to gentamicin was 68% (151/222).

Cefotaxime is a third generation antibiotics, acts upon gram-negative bacteria (Choi *et al.*, 2002). This study state that 14.2% (15/105) of clinical specimen isolates, 56.8% (33/58) of environmental, HCW source isolates and 57.6 (34/59) of cockroach source isolates were resistant to cefotaxime, other data also prove that in 2000 in Taiwan found a discrepancy in the susceptibility of *S. marcescens* to cefotaxime (resistant rate 48%) and ceftazidime (5%) (Lauderdale *et al.*, 2000). The mechanism of cefotaxime resistance in enterobacteriaceae is likely to result from the presence of β-lactomases, ESBL, Ampc β-lactamases or metallo β-lactamases (Naumiuk *et al.*, 2004). Wn *et al.* (2004), reported that 21 (62%) of *S. marcescens* isolated non-susceptible to

cefotaxime exhibited an ESBL resistant phenotype all possessed CTX-M-3 (Wn *et al.,* 2004). It will limit the choice of appropriate antimicrobial therapy for cefotaxime resistant *S. marcescens* (Hsin *et al.,* 2005). The overall resistance rate of *S. marcescens* to cefotaxime was found that 38.2% (85/222).

Chloramphenicol is an antibiotic with a broad spectrum action. It acts against both gram positive bacilli and gram negative bacilli (Bollmann *et al.,* 1989). It acquired resistance through plasmid transfer between enterobacteria and other (Lohr, 1994). In this study the clinical specimen isolates about 17.1% (18/105) environmental, HCW isolates 62% (36/58) and cockroach isolates 67.7% (40/59) were resistant to chloramphenicol. The overall resistance rate was 42.3% (94/222).

Amikacin is a widest spectrum of activity. It is recommended as a reserve drug for hospital acquired gram-negative bacillary infection (Tripathi, 2008). This study found the rate of *S. marcescens* resistance to amikacin was 51.4 % (54/105) in clinical specimen isolates, 70.6 %(41/58) in environmental, HCW isolates and 77.9 % (46/59) in cockroaches isolates, and overall resistance was 63.5 % (141/222). So, it proved that largest number of isolates were resistant to amikacin. The phenomenon of resistance to amino glycoside antibiotics occurred in early 1980s and it referred to gentamicin, tobromycin and amikacin (Echols *et al.,* 1984). Other authors also reported about *S. marcescens* developing resistance to netillimicin (Lewis *et al.,* 1983; Casewell and Ronan, 1984).

Aztreonam is an antibiotic that is active against gram-negative bacteria and gram-positive bacteria. It's a β-lactam antibiotic with a spectrum resembling amino glycosides, and its action takes place through interference in the bacterial cell wall synthesis (Naumiuk *et al.,* 2004). It's resistant to gram-negative bacteria β-lactamases, the main indication of aztreonam are hospital acquired infections originating from urinary and biliary infection (Tripathi, 2008). *S. marcescens* submitted to tests of sensitivity to aztreonam was found to be resistance about 73.8% (164/222).

Ceftazidime is most prominent feature of this third generation cephalosporin. It's highly active against pseudomonas and also active against Enterobacteriaceae. It is similar to that of cefotaxime (Tripathi, 2008). In early 1980s, 3rd generation cephalosporins were efficient in relation to most Serratia spp. However, this study obtained resistant point 48.6% (108/222) to *S. marcescens* isolates. Fast increase in the resistance to cephalosporins results from the capacity of these bacteria to produce inductive β-lactamases encoded chromosomally (Bush *et al.,* 1995). Now, its weak inducers and good substrates, in the treatment of infection by *S. marcescens* may lead to β-lactamase depression (Livemore, 1995).

Caphalothin is a first generation cephalosporin antibiotic that is characterized by its bacterial activity on gram-negative bacteria and gram-positive bacteria, by resistance to β-lactamases and sensitive to the β-lacamases producing gram-negative bacteria (Isenberg, 1992). However, *S. marcescens* isolates were resistant to caphalothin 86 % (191/222) in this study. Other reports, such as *Enterobacter* 55%, *Serratia* sp 26%, *Citrobacter* sp 14.5% and *Providencia* sp 4.5% were resistant to first and second generation cefalosporins (Casewell and Ronan, 1984).

Ciprofloxacin is one of the most potent first generation fluroquinolones active against a broad range of bacteria, the most susceptible ones is the aerobic gram-negative bacilli, especially the Enterobactriaceae (Tripathi, 2008). In this study there was 100% of susceptibility of *S. marcescens* to the ciprofloxacin antibiotic.

The profile on antimicrobial susceptibility of microorganisms isolated from the cockroaches at this hospital underlining adequate monitoring the hygiene and cleaning services, controlling and optimizing food handling, standardizing the careful use of antimicrobials and the implementation of an integrated pest controlling program (Gupta *et al.,* 1993; Khan *et al.,* 1998; Troillet *et al.,* 1999). This result can be correlated with the unrestricted use of such antimicrobials, which has enabled the emergence of resistant strains (Bollmann *et al.,* 1989). The lack of knowledge regarding hospital microbiota and the improper monitoring of antimicrobial therapeutics can lead to microbial resistance and favoring selective pressure for developing resistant strains (Wn *et al.,* 2004).

Conclusion

S. marcescens isolates were highly susceptible to ciprofloxacin antibiotic. *S. marcescens* produces different resistant spectrum

to other eight antibiotics due to multi-resistance properties. It is necessary to take them into account in microbiological diagnostics and the clinical interpretation of the result of these investigations. Based on such a viewpoint, the present study brings relevant microbiological contributions to hospital environmental sanitation related to nosocomial infection with reflection on the health care science to control the rate of morbidity-mortality at nosocomial environment.

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