Vol 2 | Issue 1 | Jan –Jun 2011 | 7-11. **Print ISSN – 2229 7502**

Research Article

ANTIMICROBIAL SENSITIVITY PROFILE OF *SERRATIA MARCESCENS* STRAINS ISOLATED IN GOVERNMENT GENERAL HOSPITAL, NAGAPATTINAM, TAMILNADU, INDIA.

[1]S. Sethuraman, [2]A. Arunachalam, [3]M. Karthikeyan, [3]S. Ashhutosh Kumar, [3]S. Manidipa, [4]R. Senthilraj

[1]Department of chemistry, SCSVMV University, Kanchipuram, Tamilnadu, India..
[2]Department of Pharmaceutics, Sasikanthreddy College of Pharmacy, Nellore, Andhrapradesh, India.
[3]Department of Pharmaceutics, AKRG College of Pharmacy, West Godavari, Andhrapradesh, India.
[4]Department of pharmaceutical biotechnology, Vikas College of pharmacy, Jangaon, Warangal, Andhrapradesh, India.

ABSTRACT

Serratia marcescens is one of the nosocomial pathogen, it cause secondary infections like urinary, respiratory, wound, sinusitis, septic arthritis and peritonitis in the hospitalized patients. *S. mancescens* have been developing resistant to majority of antibiotics due to acquiring genetic resistance, indiscriminate and empirical uses of antibiotics causes selection of resistant strains. In our study we used totally 222 *S. marcescens* isolate strains for testing antibiotic sensitivity using ampicillin, gentamicin, cefotaxime, chloramphenicol, amikacin, aztreonam, ceftazidime, cephalothin, and ciprofloxacin. Through the antibiotic disc method the sensitivity was analysed in the presence of zoon of inhibition around the antibiotic disc. In our result 222 strains of *S. marcescens* gave 100% of susceptible to ciprofloxacin and it gave different resistant spectrum to the other 8 antibiotics. It confirmed ciprofloxacin give 100% of recovery to *S. marcescens* infections.

Keywords: *Serratia marcescens,* Antimicrobial, Ampicillin, Gentamicin, Cefotaxime.

INTRODUCTION

The antimicrobials in the therapeutics of infectious diseases were described over 2,500 years ago. At that time, they were regarded as the solution to all diseases caused by microorganisms [1]. *S. marcescens* has been recognized as the cause of many hospital epidemics [2-3] and a causative agent of hospitalized infection (nosocomial infection) [4]. It causes several diseases as a secondary infection such as urinary, respiratory, wound and septic arthritis, peritonitis and sinusitis [5]. *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 [6]. However, their widespread, indiscriminate and empirical uses of antibiotics has 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 [1]. 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.

MATERIALS AND METHODS

SEED CULTURE PREPARATION

In our previous objectives we isolated 105 *S. marcescens* strains from patient clinical samples, 58 strains of *S. marcescens* from environmental & Health care workers isolates and 59 strains from cockroaches got from hospital environment. Totally 222 *S. marcescens* isolates were used for antimicrobial sensitivity tests using the disc diffusion method, according

*Corresponding Author **S. Sethuraman** E mail: harisarun1985@gmail.com

to the National Committee for Clinical Laboratory standards (NCCLS) recommendations for the determination of antimicrobial susceptibility [7]. All 222 strains were seeded in agar slants and incubated at 37^0C 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

After the broth inoculates preparation in 0.8% sterile saline solution, the strains were seeded in Muller Hinton agar, the antibiotic disc were distributed in an equidistant fashion and the plates were incubated at a temperature of 37^0C for 24 hours. The antimicrobial agents used were ampicillin (10µg), gentamicin (10µg), cefotaxime (30µg), chloramphenicol (30µg), amikacin (30µg), aztreonam (30µg), ceftazidime (30µg), cephalothin (30µg) and ciprofloxacin (5µg). The susceptible and resistance isolates were identified in the presence of zone of inhibition around the antibiotic discs.

RESULTS:

Clinical specimen isolates strains

105 strains of *S. marcescens* obtained from patient's clinical specimen were tested for antimicrobial susceptibility (Table 1).57.1% (60/105) to gentamicin, 14.2% (15/105) to cefotaxime, 17.1% (18/105) to chloramphenicol, 51.4%(54/105) to amikacin, 65.7% (69/105) to aztreonam, 23.8% (25/105) to ceftazidime, 85.7% (90/105) to caphalothin and 0% (0/105) to ciprofloxacin.

Environmental & HCW specimen isolate strains

Totally 58 *S. marcescens* strains of environmental source isolates were submitted for antimicrobial susceptibility (Table 2).

Table 2 shows the frequency of environmental & HCW isolates of *S. marcescens* in relation to antimicrobial susceptibility 84.4% (49/58) were found to be resistance to ampicillin, 74.1%(43/58) to gentamicin, 56.8%(33/58) to cefotaxime, 62.0%(36/58) to chloramphenicol, 70.6%(41/58) to amikacin, 77.5%(45/58) to aztreonam, 67.2%(39/58) to ceftazidime, 82.7% (48/58) to caphalothin and 0% (0/58) to ciprofloxacin.

Cockroaches isolate strains

59 strains of *S. marcescens* from cockroaches in hospital environmental were tested for its antimicrobial susceptibility (Table 3).

Table 3 shows the frequency of antimicrobial susceptibility of *S. marcescens* strains. 94.9% (56/59) were found to be resistance to ampicillin, 81.3%(48/59) to gentamicin, 57.6%(34/59) to cefotaxime, 67.7%(40/59) to chloramphenicol, 77.9%(46/59) to amikacin, 84.7%(50/59) to aztreonam, 74.5%(44/59) to ceftazidime, 89.8% (53/59) to caphalothin and 0% (0/59) to ciprofloxacin.

Figure 1, demonstrates drug sensitivity of 105 *S. marcescens* strains isolated from clinical specimens (A), 58 *S. marcescens* strains from environmental & HCW sources (B) and 59 *S. marcescens* strains from cockroaches in hospital environment (C). The data suggest that all the study strains were susceptible only to ciprofloxacin antibiotic. The sensitivity of analyzed *S. marcescens* strains to the antibiotics was given on Figure 1.

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

Total no. of strains	Antibiotic	No. of resistant	No. of susceptible	% of resistant
105	Am	93	12	88.5
105	Gn	60	45	57.1
105	Ctx	15	90	14.2
105	Chl	18	87	17.1
105	An	54	51	51.4
105	Azt	69	36	65.7
105	Caz	25	80	23.8
105	Cap	90	15	85.7
105	Cip	0	105	0

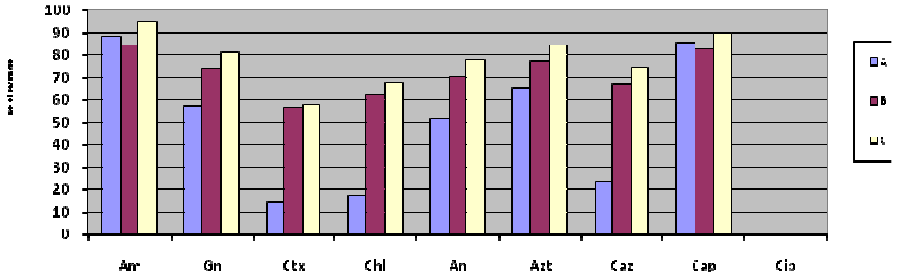
Table 2.Environmental & HCW specimen isolates of *S. marcescens* antimicrobial sensitivity

Total no. of strains	Antibiotic	No. of resistant	No.of susceptible	% of resistant
58	Am	49	09	84.4
58	Gn	43	15	74.1
58	Ctx	33	25	56.8
58	Chl	36	22	62.0
58	An	41	17	70.6
58	Azt	45	13	77.5
58	Caz	39	19	67.2
58	Cap	48	10	82.7
58	Cip	0	58	0

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

Total no. of strains	Antibiotic	No. of resistant	No. of susceptible	% of resistant
59	Am	56	03	94.9
59	Gn	48	11	81.3
59	Ctx	34	25	57.6
59	Chl	40	19	67.7
59	An	46	13	77.9
59	Azt	50	09	84.7
59	Caz	44	15	74.5
59	Cap	53	06	89.8
59	Cip	0	59	0

Figure 1. 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.
A - Clinical isolates strains - 105
B - Environmental isolates strains - 58
C - Cockroach isolates strains - 59

DISCUSSION

The findings in this study seem to confirm the ciprofloxacin is an effective antibiotic to treat the *S. marcescens* infected patients. In the last decade increased resistance to antibiotics used in hospitals was also observed with respect to *S.* marcesce [8]. The majority of strains proved resistance to many antibiotics. Among our study *S. marcescens* strains, there was a large percentage of strains which were resistance to ampicillin and also reported by many authors [12]. Ampicillin is clinically useful due to the inhibition of β-lactamase, which is effective in the treatment of serious infection such of respiratory [13], urinary [10], gynecological and septicemia triggered by β-lactamase producing organisms [9].

The results of our study confirm the resistance rate of *S. marcescens* were submitted to ampicillin 85.5% (93/105) of clinical specimen isolate strains, 84.4 %(49/58) of environmental isolate strains and 94.9% (56/59) of cockroach isolates strains. 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. However in our study *S. marcescens* isolated from the clinical specimen 57.1 % (60/105) were resistance the environmental sources isolate 74.1% (43/58), and *S. marcescens* isolated from the cockroaches 81.3% (48/59) were showed resistance to gentamicin. Many other authors also issued reports about the penomenion of resistance to aminoglycoside antibiotics gentamicin [15]. The enterobacteria isolates from the cockroaches were relatively resistant to gentamicin; 1.5% for *K. pneumoniae*, 14% for *E. aerogenes*, 13% for *S. marcescens* [15]. The overall resistance rate of *S. marcescens* to gentamicin was 68% (151/222).

Cefotaxime is a third generation antibiotics, acts upon gram-negative bacteria [16]. This study and that 14.2% (15/105) of clinical specimen strains, 56.8% (33/58) of environmental source strain and 57.6 (34/59) of cockroach source strains, 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%) [17].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 [18]. The *S. marcescens* isolated non-susceptible to cefotaxime exhibited an ESBL resistant phenotype all possessed CTX-M-3 [19]. It will limit the choice of appropriate anti microbial therapy for cefotaxime resistance *S. marcescens* [20]. 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 [24], in our study clinical specimen isolate strains about 17.1% (18/105) environmental isolate strains 62% (36/58) and cockroach isolate strains 67.7% (40/59) were resistant to chloramphenicol. The overall resistance rate was 42.3% (94/222). It acquired resistance through plasmid transfer between enterobacteria and other [12].

Amikacin is a widest spectrum of activity. It is recommended as a reserve drug for hospital acquired gram-negative bacillary infection [14]. In our study we found the rate of *S. marcescens* resistance to amikacin was 51.4 %(54/105) in clinical isolate strains, 70.6 %(41/58) in environmental isolate strains and 77.9 %(46/59) in cockroaches isolate strains, and overall resistance was 63.5 %(141/222). So, it proved that largest number of strains were resistant to amikacin. The phenomenon of resistance to aminoglycoside antibiotics occurred in early 1980s and it referred to gentamicin, tobromycin and amikacin [21]. Other authors also reported about *S. marcescens* developing resistance to netillmicin [22-23].

Aztreonam, an antibiotic that is active against gram-negative bacteria and gram-positive bacteria and entrobacteria. It's a β-lactam antibiotic with a spectrum resembling aminoglycosides, and its action takes place through interference in the bacterial cell wall synthesis [18]. It's resistant to gram-negative bacteria β-lactamases, the main indication of aztreonam are hospital acquired infections originating from urinary and biliary infection [14]. *S. marcescens* submitted to tests of susceptibility to aztreonam was found to be resistance about 73.8% (164/222).

Ceptazidime is most prominent feature of this third generation cephalosporin. It's highly active against pseudomonas and also active against Enterobacteriaceae is similar to that of cefotaxime [14]. In early 1980s, 3rd generation cephalosporin were efficient in relation to most Serratia spp. However, obtained results point 48.6% (108/222) of activity of these antibiotics with respect to *S. marcescens* strains. Fast increase in the resistance to cephalosporin results from the capacity of these bacteria to produce inductive β-lactamases encoded chromosomally [25]. Now its weak inducers and good substrates, in the treatment of infection by *S. marcescens* may lead to β-lactamase depression [10].

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 [13]. However, the results of tests of susceptibility to that antibiotics are 86%(191/222) of S. marcescens strains were resistant to caphalothin, the 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 cephalosporin [23].

Ciprofloxacin is one of the most potent first generation fluroquinolones active against a broad range of bacteria, the most susceptible ones are the aerobic gram-negative bacilli, especially the Enterobactriaceae [14]. In our study there was 100% of sensitivity of *S. marcescens* to the ciprofloxacin antibiotic.

CONCLUSION

Nine antimicrobials were used in our study, the demonstrated all isolates were resistance to eight antimicrobials. 100% of susceptibility to one antimicrobial drug. This result can be correlated with the unrestricted use of such antimicrobials, which has enabled the emergence of resistant strains [24]. The lack of knowledge regarding hospital microbiota and the improper monitoring of antimicrobial therapeutics can lead to microbial resistance and favoring selective pressure to developing resistant strains.

REFERENCES

1. Riberro Filho N. agents Antimicrobials, In: Fernander A.T et al., Infectious hospital area interfere with diseases. opaulo: Guanabara Koogan. 1, 2000, 1485-1534.
2. Farmer JJ 3ed, Davis BR, Hickman FW, Presley DB, Bodey GP, Negut M, et al., Detection of Serratia outbreaks in hospital. *Lancet,* 2, 1976, 455-459.
3. Worevehomang WW, Wang LS, Cheng DL, Lin SJ, Chin TD, Hinthorn DR, et al., *Serratia marcescens* bacteremia. *J Formes Med Assoc*., 90, 1990, 88-93.
4. Yu VL. *Serratia marcescens*: historical perspective and clinical review. *N Engl J Med.,* 300, 1979, 887-893.

5. Eisenstein BI, Zaleznik DF. Enterobactria. In: mandell GL, Benett JE, Dolin R, eds. Principles and practice of infectious diseases. 5th ed. Vol 2. Philadelphia: Churchill Livingstone: 2000, 2294-2310.
6. Bennett PM, Chopra I. Molecular basis of beta-lactamases induction in bacteria. *Antimicrob agent's chemother.,* 37, 1993, 153-158.
7. National committee for clinical laboratory standards (NCCLS). Performance standards for antimicrobial susceptibility testing, Approved standard M2-A8 and M7-A6. Wayne. Pa: National committee for clinical laboratory standards. 2004.
8. Bollmann R, Halle E, Sokolowska-Kohel W, Granel EL, Buchhilz P, Klane I: nosocomial infection due to Serretia marcescens – clinical finding, antibiotic susceptibility patterns and fine typing. *Infection*, 17, 1989, 294-300.
9. Haddy RJ, Mann BL, Nadkanii DD, Guz RF, Elshoff DJ, Buendia FC; Nosocomial infection in the community hospital: Sever infection due to Serratia species. *J Fam Pract*, 42, 1996, 273-277.
10. Livemore DM: β-lactamases in laboratory and clinical resistance. *Clin Microb Rev,* 8, 1995, 557-584.
11. Jarvis WR, Martone WJ: Predominant pathogens in hospital infections. *J Anti Chem,*. 29, 1992, 19-24.
12. Lohr JA, Downs SM, Dudley S, Donowitz LG: Hospital acquired urinary tract infections in the paediatric patient: a perspective study. *The paediatric infectious diseases Journal*, 13, 1994, 8-12.
13. Isenberg HD: Enterobactriaceae: The genus Serratia.in: Gorbach SL, Bartlett JG, Blacklow NR: Infectious diseases. WB. Saunders Company, Philadelphia, 1992, 1473-1474.
14. Tripathi KD, MD. Essentials of medical pharmacology, Jaypee Brothers medical publications (P) LTD, 2008, 667-726.
15. Zhang Y: A two years prospective survey on nosocomial infections. Chung-hua, Hsueh Tsa Chin (Chines medical journal) (Taipei). 71, 253-256.
16. Choi SH, Kim YS, Chung JW, Kim TH, Choo EJ, Kin MN, et al., Serratia bacteremia in a large university hospital: trends in antibiotic resistance during 10 years and implications for antibiotic use. *Infect control Hosp Epidemiol*., 23, 2002, 740-747.
17. Lauderdale TL, Clifford Mc Donaldl, Shiau YR, Chen PC, Wang HY, Lai JF, et al., The status of antimicrobial resistance in Taiwan among Gram-negative pathogens: the Taiwna surveillance of antimicrobial resistance (TSAR) program, 2000. *Diagn microbial infec Dis*., 48, 2004, 211-219.
18. Naumiuk L, Baraniak A, Gaiadkourski M, Krawozyk B, Rybak B, Sadowy E, et al., Molecular epidemiology of Serratia marcescens in two hospitals in Gdansk, Poland, over a 5-year period. *J clin Micribiolo*., 42, 2004, 3108-3116.
19. Wn LT, Tson MF, Wu HJ, Chen HE, Chuang YC, Yu WL. Survey of CTX-M-3 extended spectrum beta-lactamase (ESBL) among cefotaxime- resistant Serratia marcescens at a medical center in middle Taiwan. *Diagn Microbial Infect Dis.,* 49, 2004, 125-129.
20. Hsin I shin, Hsin-Chun lee. Serratia marcescens bacteremia at a medical center in southern Taiwan: high prevalence of cefotaxime resistance. *J Microbiol Immunol Infect.,* 38, 2005, 350-357.
21. Echols RM, Palmer DL, King RM, Long GW: Multidrug resistant Serratia marcescens bacterimia related to urologic instrumentation. *South Med J.,* 77, 1984, 173-177.
22. Lewis DA, Hankey PM, Watts JA, Spellen DC, Primavesi RJ, Fleming PJ: infection with netilimycin resistant Serratia marcescens in a special care baby unit. *Br med J cli Res Ed,* 287, 1983, 1701-1705.
23. Casewell MW, Ronan P: Infection with netilimycin resistant Serratia marcescens. *Br med J cli Res Ed*, 1984, 287-288.
24. Bollmann R, halle E, Sokolowska-kohten W, Granel EL, Buchholz P, Klave I: Nosocomial infection due to Serratia marcescens-clinical findings, antibiotics susceptibility patterns and fine typing. *Infection,* 17, 1989, 294-300.
25. Bush k, Jacoby GA, Medeiras AA: A functional classification scheme for β- lactmases and its correlation with molecular structure. Antimicrob *agents Chemother*, 39, 1995, 1211-1233.