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Antibiotic Sensitivity Pattern of *Serratia Species* from Clinical Samples at a Tertiary Care Hospital in Rawalpindi

Rafia Irfan, Iqra Sadiq, Amna Amer, Irfan Ali Mirza, Faisal Hanif, Wajid Hussain

ABSTRACT

Objective. To determine the antibiotic resistance profile of *Serratia spp* isolated from various clinical specimens.

Study Design and Settings: A descriptive cross-sectional study on antibiotic resistance profile of *Serratia spp* isolated from various clinical specimens was carried out in the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from 1st July 2017 to 30th June 2021.

Methodology: 464 clinical specimens yielding growth of *Serratia spp* were included in the study. *Serratia spp* were identified by using Standard Microbiological procedures. Modified Kirby Bauer disc diffusion method was used for Antibiotic Susceptibility testing. The clinical data was analyzed prospectively from July 2017 to June 2021 for a period of 4 years. The spectrum of diseases caused by *Serratia spp* along with resistance profiles were analyzed. . Data obtained was analysed using SPSS 24.

Results: High yield of this bug was obtained from pus and tissue specimens 150 (32%).130(28%) isolates were retrieved from blood cultures, whereas respiratory specimens contributed to 89(19 %) isolates of *Serratia spp.* According to the antimicrobial susceptibility pattern, 154 (33.3%) isolates were sensitive to Meropenem, 150(32.2%) were susceptible to Doxycycline and 118 (25.5%) to Amikacin, making them the preferred antibiotics to be used in our setup.

Conclusion: *Serratia marcescens* isolates are increasingly resistant to antibiotics. Clinical isolates of *Serratia* exhibited highest resistance to Ciprofloxacin, Ceftriaxone, Gentamicin and Piperacillin/tazobactam.

Keywords: Antimicrobial, Neonatal Intensive care Units, Outbreaks, *Serratia*

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INTRODUCTION:

The genus *Serratia* belongs to family Enterobacteriaceae is Gram negative, motile, nonspore forming rod and is a facultative anaerobe. This bacterium was firstly discovered in 1819 by Bizio, he identified it as a cause of the bloody discoloration on cornmeal mush. He gave name to the organism in honour of the Italian physicist, Serratia who invented the steam boat. *Serratia marcescens* was originally thought to be an innocuous, non-pathogenic, saprophytic water organism, moreover it was often used as a biological marker because of its easily disguisable red colored colonies. *Serratia marcescens* produces a red pigment called prodigiosin. Prodigiosin was used as a dye for different textiles as well as for materials used for medical purposes. Serratia being ubiquitous in nature, it is widely distributed in environment including soil, water, insects, animals and plants.[1]To date, 14 species of *Serratia* have been identified, out of which eight are associated with human infection. Among these *Serratia marcescens, Serratia liquefaciens and Serratia odorifera* are the most important ones. *Serratia rubidaea* is also encountered though infrequently. *S. marcescens* is the most commonly isolated specie in the laboratory amongst all other species of this genus .[2]The presence of *Serratia* species in hospital environment such as medical equipment, lotions, antiseptics, medications, blood products and sinks, water supplies and instruments potentiate its ability to cause hospital acquired infections.

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The contaminated hands of Health care workers are an important mode of spread of this bacterium. The organism does not lead to primary invasive diseases, but it causes an infection when it gets access to an appropriate immunocompromised host. *Serratia* species harbor few virulence mechanisms thus it is considered to be an opportunistic pathogen. However, *Serratia spp* can also cause community acquired infections. The spectrum of infections varies from respiratory tract infections, intraabdominal infections urinary tract infections, skin and soft tissue infections, osteomyelitis, meningitis to bacteremia and sepsis. Thus, it is a notorious bug in microbiological world. Various risk factors which are associated with these infections are: Infants with low birth weight, prolonged immunosuppressive therapy, prolonged intake of antimicrobials, prolonged hospital stay, indwelling catheters like possession of central venous catheters, urinary catheters, mechanical ventilatory apparatus, respiratory tract instrumentation and various underlying diseases such as solid organ and hematologic malignancy, chronic pulmonary disease, and diabetes mellitus.[3]

In hospital settings, it has the propensity to cause outbreaks due to its presence in hospital environment, mostly in neonatal Intensive Care Units.[4,5.] Neonates are more prone to the infections by organisms owing to their immature immune system.[6] The most common site of infection in these cases is blood stream, followed by the respiratory and gastrointestinal tract. The high incidence of S. marcescens bacteremia in neonates is mostly associated with gut colonization during the first 3 days of life. In immunocompromised patients, there are reported cases of skin and soft tissue infections such as granulomatous ulceration, abscess, bullous cellulitis, and necrotizing fasciitis, which further explains this isolate being opportunistic in nature.[7] The mortality rates are higher in cases of septicemia, meningitis and endocarditis caused by *Serratia species.*[8] The effective diagnosis and treatment of these infections is crucial as this bacterium can exhibit dual antimicrobial resistance ability that is by both intrinsic mechanisms and acquired antimicrobial genes. The acquired resistance is for beta-lactam antibiotics, aminoglycosides and quinolone group of antibiotics whereas natural resistance is also there to many antimicrobials including narrow spectrum penicillins, aminopenicillins, amoxicillin-clavulanate, numerous cephalosporins, colistin and nitrofurantoin.[9] This ability of being multidrug resistant, makes this isolate difficult to be treated and eliminated from the colonized sites. So, clinicians have to tailor the empirical choices for this bacterium according the antibiogram of the hospital or the area whichever is available.

Serratia sp particularly *Serratia marcescens* causes a variety of local and systemic infections in both healthy and immunocompromised host. This facultative anaerobe poses an impending threat to patients admitted in intensive care units by causing outbreaks. Poor infection control practices and irrational use of broad-spectrum antibiotics are major contributing factors behind such outbreaks.[10] The frequency of *Serratia species* as well as the susceptibility pattern of this bacterium has not been studied in our setup. To fill these gaps in knowledge about this important microorganism, we studied the trends of isolation and antimicrobial susceptibility patterns of *Serratia* species in our setup. The rationale of this study was to determine frequency, distribution and antibiotic susceptibility profile of *Serratia spp.* which will help the clinicians to make choices for empirical treatment of nosocomial infections suspected to be caused by *Serratia species*. This knowledge can help in reducing morbidity and mortality in intensive care units especially neonatal ICUs. It can be helpful in establishing antimicrobial stewardship to minimize unnecessary use of antibiotics, which may prevent emerging antimicrobial resistance.

METHODOLOGY:

The department of Microbiology Armed Forces Institute of Pathology, Rawalpindi receives clinical specimens submitted from Combined Military Hospital (CMH) Rawalpindi, Military Hospital (MH Rawalpindi), Armed Forces Bone Marrow Transplant Center (AFBMTC), Armed Forces Institute of Urology (AFIU) and Army Liver Transplant Unit (ALTU). The clinical data was analyzed prospectively from July 2017 to June 2021 for a period of 4 years after taking permission from Ethical Committee of our institute (Ethical Review Board number 479). All *Serratia spp* isolated during the study period were included, however repeat specimen from the same patient yielding *Serratia* specie with similar antibiogram was excluded. *Serratia specie* were identified by its phenotypic and biochemical characteristics using Standard Microbiological procedures. All the specimens were inoculated on Blood agar and MacConkey agar. The culture plates were incubated at 37*c for 24 to 48 hours. Colony morphology particularly pigment production was noted. Modified Kirby Buer disc diffusion method was used for Antibiotic Susceptibility testing for the recommended Antibiotics by Clinical Laboratory Standard Institute.[11] *Eschrechia coli* ATCC 25922 was used as control organism. The spectrum of diseases caused by *Serratia spp* along with resistance profiles and other demographic data were analyzed over the described period. Data obtained was analysed using SPSS 24. Demographic data was assessed using descriptive statistics. Mean and standard deviation (SD) were calculated for numerical variables, like age. Categorical variables were expressed using frequencies and percentages. $P<0.05$ was considered statistically significant.

RESULTS:

Serratia species were identified in 464 of clinical specimens from July 2017 to June 2021. The frequency of isolation of *Serratia species* is shown in Figure 1. Out of total 464 isolates, 329 were isolated from male patients and 135 from

female patients, Male to female ratio of 2.43:1. The age wise distribution of patients in which *Serratia* species were isolated is shown in Figure2. The distribution of *Serratia* species according to specimen type which includes respiratory, pus and tissue, blood, urine, fluids is depicted in Figure 3. According to the antibiotic susceptibility tests, 325 (70%) isolates were resistant to ciprofloxacin while only 117 (25.2%) were resistant to Amikacin. The percentage of isolates resistant to other antibiotics is shown in figure 4.

DISCUSSION:

The frequency of *Serratia* species in various clinical specimens was studied. A total of 464 isolates were isolated over four-year time period from July 2017 to June 2021.There was surge in number of *Serratia spp* in years 2017 and 2019 due to outbreaks in Neonatal Intensive Care Units. In first six months of year 2019, highest number of 98 cases of

Figure 1: Six monthly distributions of cases of infection caused by *Serratia spp*

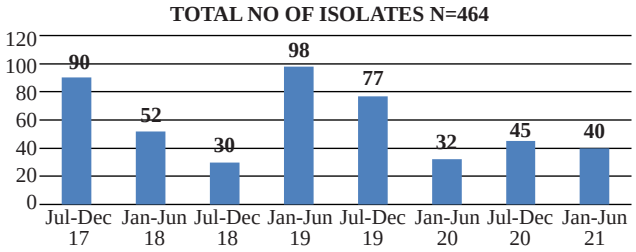


Figure 2: Total number of *Serratia* species isolated in patients of various age groups.

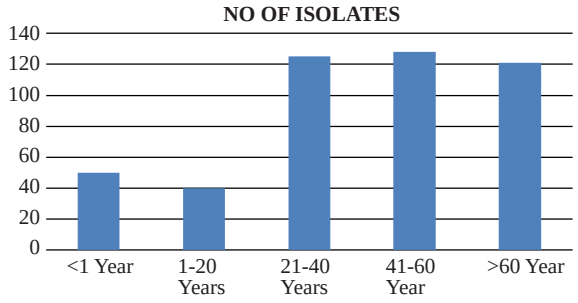


Figure 3: Frequency of isolation of Serratia spp from various clinical specimens

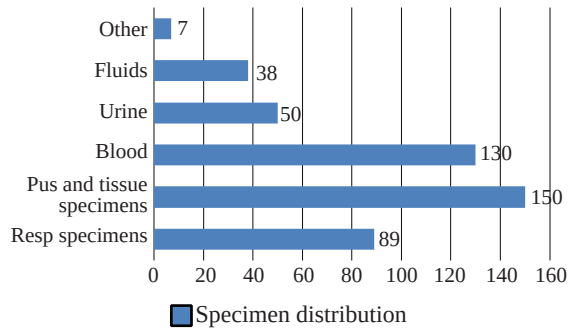
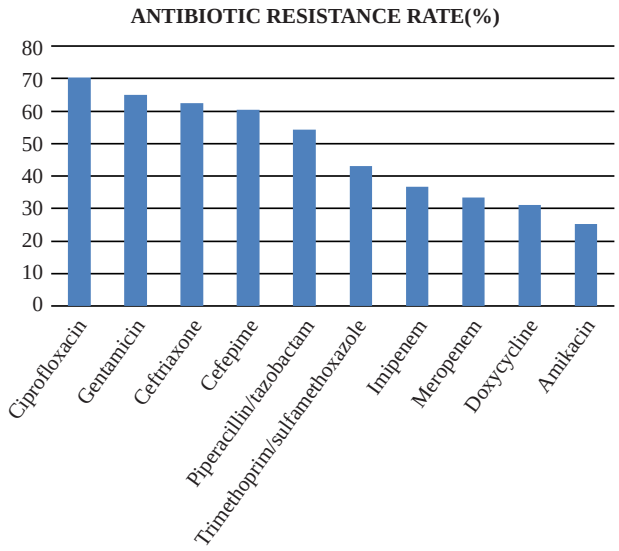


Figure4: Antibiotic resistance rates of *Serratia* species isolated from various clinical specimens



Serratia were isolated. In remaining years, it was sporadic. The baseline rate of infection or colonization due to *Serratia spp* in our setup was noted to be 7 to 8 cases/month which was in contrast to study done in Argentina in which the sporadic cases of *Serratia spp* infections were 1-4 cases /month in 2005 and 2006. In a similar study during the outbreak in 2007 to 2008, the rate reached 10 cases /month.[12.] It is comparable with our study being 11 and 10 cases /month in outbreak years.

In our institute outbreaks of this isolate occurred in Neonatal Intensive Care Units only. This finding is supported by various studies mentioned in literature as the bug is notorious to cause outbreaks in Neonatal and Pedriatic intensive care units. Lot of reported outbreaks occurred due to non-adherence to standard infection control and prevention practices. A study conducted by Cristina et al in February 2019 in Italy gave emphasis on outbreaks by *Serratia spp* in Neonatal Intensive Care Unit. The report urged that prompt enforcement of handwashing and application of contact-based precautions are essential factors in halting the spread of this pathogen.[10] Moreover a study published in Frontiers of microbiology by Claudia Saraleguei et al also highlighted the same issue that precautions taken in neonatal Intensive Care Units can prevent the infection by this isolate.[13]

We found out that male patients were much more commonly infected/ colonized with *Serratia spp* while clinical samples of female patients yielding *Serratia spp* were quite few in number. Although there is no plausible reason as to why this isolate is significantly affecting more males but our observation equates with Morillo *et al* who reported 13 male (72.2%) and 5 female children (27.8%) were having infection caused by *Serratia spp.*[14]

The present study revealed that Isolation of *Serratia species* was highest among persons aged between 40-60yrs (27.58%)

and lowest incidence was in age group 1-20years (8.62%). If we compare with other studies, Ferreira et al in Brazil in 2020 determined the prevalence of *Serratia* by age which unfolded to be 0–1 day (12.96%), 18-59 years (38.89%), 60 years or more (48.15%), the highest being more than 60 years age and lowest 1-20 years of age.[15] This is like some other studies that showed advanced age male patients as presenting a higher risk of contracting *Serratia* infections.[16-18]

In our study, Pus and Tissue specimens yielded maximum number of *Serratia Specie* which was then followed by blood culture samples, respiratory samples and urine. Sterile fluids including cerebrospinal fluid also accounted for the growth of this dreadful pathogen. Umblical venous catheter tips, Central venous catheter tips and biliary stent tips were the specimens included in "other" category and also yielded growth of *Serratia spp*. These findings suggest maximum cases having *Serratia specie* infection were of skin and soft tissue. Outbreaks of Wound infections particularly post operative surgical site infections have also been mentioned in literature.[19] *Serratia marscenes* is also an etiology of Bacteremia, Sepsis and Urinary Tract infections. In our present write up during outbreak years *Serratia* specie were most commonly obtained from Blood cultures. This finding is analogous with a study published in Journal of Environmental research and Public health by Christina et al in 2019.[20]A retrospective study done in General hospital in Nigeria in year2019, revealed *Serratia Spp* from Respiratory specimens including sputum (38%),[21] which is much higher observation from our narration.

We also analyzed the Antibiotic susceptibility testing Data obtained from the electronic system of our Laboratory on *Serratia species* isolated in the defined time period. The antibiotic with the highest resistance rate was found to be Ciprofloxacin (70.2%). Antibiotic with the lowest resistance rate was determined as Amikacin (25.2%). In one study of India[8], however the resistance rate of these two drugs were quite different, Ciprofloxacin having 14.3% resistance while Amikacin having 71.4% resistance rate. This can be due to difference in use of these drugs in empirical treatment in both set ups which can lead to development of resistance in these areas.

If we analyze Cephalosporins sensitivity rate (particularly ceftriaxone) we see them to be having a very high resistance profile in our study (62.3%). The high proportion of isolates resistant to this beta lactam antibiotic was also observed from a study conducted in Turkey in 2018 by Simsek et al, which showed ceftriaxone to be the antibiotic with the highest resistance rate (22.7%).[21]

In our study, Carbapenems more specifically imipenem had low resistance rates (36.7%) as compared to most of other drugs, which is comparable to the study done by Hayashi et al in June 2021 in which all the isolates included in study were sensitive to Carbapenems.[22] The work done by Xu Q et al in Tertiary hospital in China in year 2020 also supported our study findings by demonstrating Amikacin to be the most sensitive drug.[23]Aminoglycosides were also found to be most sensitive for this bacterium in study done by Ferreira et al. [15]An extensive 8-year study done in Taiwan, revealed Ceftazidime and Imipenem with consistently high susceptibility rates to *Serratia species* while ciprofloxacin had highest resistance rate.[24]

Our study showed that isolates of *Serratia* Spp had high resistance to ciprofloxacin, gentamicin, ceftriaxone, cefepime and pipercillin/tazobactum. However, meropenem, imipenem, trimethoprim sulfamethoxazole, doxycycline and amikacin were found to be the most suitable antibiotics for treatment. We suggest Carbapenems particularly imepenem as agents for empirical treatment till the availability of susceptibility results. Amikacin though being most sensitive of all antibiotics should not be used as monotherapy and hence for empirical treatment.

The limitation of our study was there is no follow up data available which can explain the morbidity and mortality of the patients having infection by these strains of *Serratia spp.* Thus, the effectiveness of culture directed antimicrobial therapy to eradicate this pathogen was also not ascertained. Moreover, no clinical history correlation was done to ascertain the status of these strains being actual pathogen or colonizers.

CONCLUSION:

Our present account concluded that *Serratia* species in clinical isolates are increasingly resistant to antibiotics. The clinical isolates of *Serratia* specie exhibited highest resistance to ciprofloxacin, ceftriaxone, gentamicin and piperacillin / tazobactam. Carbapenem particularly imipenem and aminoglycoside particularly amikacin were least resistant antimicrobials, which fulfilled our objective to determine the antibiotic resistance profile of this notorious bacteria.

Authors Contribution:
Rafia Irfan: Idea and Concept
Iqra Sadiq: Sample Collection
Amna Amer: Sample collection and process
Irfan Ali Mirza: Process and analysis
Faisal Hanif: Writing, reviewing and conclusion
Wajid Hussain: Statistics and discussion

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