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Research article

Serratia Marcescens outbreak on a general pediatric ward in Benin

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Abstract:

Background: Increasing reports in the literature document the existence of nosocomial transmission of *Serratia marcescens.* The consequences of infections associated to this bacterium can be severe, so it was important to establish strategies for prevention. This study aimed to provide the prevalence of this bacterium in a hospital environment and to examine the main factors increasing the risk of cross contamination.

Methods: Various specimen obtained from 790 hospitalized children aged from 0 to 7 years were examined and 940 others samples taken from hands of medical personnel, and various hospital surfaces were also screened for *Serratia marcescens* presence. Susceptibilities to antimicrobial agents were tested by the disk diffusion method according to NCCLS guidelines. Aggressive infection control measures were instituted.

Results: About 123 (**38%**) patients were infected by *Serratia marcescens*, including septicaemia 65 (**52.85%**), 31 (**25.2%**) urinary tract infections, 12 (**9.75%**) pneumonia and 15 (**12.2%**) others infections. From hospital environment, 108/940 (**11.50%**) isolates were obtained. Antimicrobial susceptibility testing revealed that 56% of strains displaying multi resistance. Infected patients were cohorted and placed on contact precautions. Investigation by the infection control team revealed that the distributors of antiseptic were the main path of *Serratia marcescens* dissemination.

Conclusions: A substantial proportion of cases appeared to be of medical devices origin. New infection control policies and engineering plans were initiated on the basis of our results. Antimicrobial resistance is particularly harmful to infectious disease management in low-income countries since expensive second-line drugs are not readily available.

Key words: Benin, Nosocomial infection, prevention, pediatrics unit, *Serratia marcescens*.

Introduction:

Serratia marcescens (S. marcescens) is a facultative anaerobes Gram-negative bacillus, classified in the tribe *Klebsielleae* of the large family Enterobacteriaceae. The organism has been recognized as an opportunistic pathogen in humans since 1960[1,2]. However the role of the organism as a nosocomial pathogen has been cause appreciated more recently[3,4]. The bacterium is commonly found in soil, water, plants, and animals, is widely present in non-potable water in developing countries due to poor chlorination[5,6].The clinical relevance of *S. marcescens* in humans appeared to be increasing since the bacterium has been associated with various infections such as endocarditis, septic shock, urinary tract infections, osteomyelitis[7-9]. This bacterium is resistant to many antibiotics traditionally used to treat bacterial infections and has been identified as responsible of large outbreaks in pediatric unit[1,5,10]. Patients can be contaminated from various devices in hospital environment[2,11]. In a secondary hospital (Zou/Collines Department Hospital Center)

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located in Abomey, Benin, an increase in the number of cases of infections associated to *S. marcescens* was observed over the past years, with unusual accumulations of isolates in the pediatric unit. The purpose of this study was to examine the resistance in this bacterium and the main factors increasing the risk of cross contamination.

Material and methods

Patients and sampling

A six-month surveillance was carried out at the pediatrics in-patient department of the secondary hospital (Zou/Collines Department Hospital Center) located in Abomey, Benin, from June 15th to December 15th, 2009. Children aged >3 months to <7 years were enrolled consecutively after they had completed consultation with the physician on duty. Informed verbal consent was obtained by parents or guardians. We examined various specimen obtained from these children who developed nosocomial infection during this period. These patients were studied as part of a prospective evaluation of nosocomial acquisition and transmission of *S. marcescens.*

The samples were performed in the all six rooms of the pediatric unit. Specimens were collected from the hands of nursing staff, medical devices, various hospital surfaces and inanimate objects such as, liquid antiseptic at sinks, bed rails, medication carts, urinary catheters and urine bags, disinfectants, nurse call buttons, stands for infusion apparatus, intravenous pump buttons. The samples were weekly taken with sterile cotton tipped swabs moistened with phosphate buffered saline (pH 7.2) and transported to the laboratory immediately.

Strain identification

Sample have been preliminary cultured on Deoxyribonucleic acid, Toluidine blue, Cephalotin agar, (DTC) as recommended by Freney *et al.*[12]. The final identity was determined with API 20E strips (bio Mérieux). *S. marcescens* strains have been primarily investigated for morphological and biochemical characteristics including, the production of a pigment called prodigiosin. Other enteropathogenic bacteria have also been checked. Only samples in which *S. marcescens* were at pure/major culture of aerobic flora (90% UFC) have been considered for further investigation.

Antimicrobial susceptibility testing

Susceptibility testing was performed by the disk diffusion method on Mueller-Hinton (Bio-Rad-Diagnostic Pasteur, Marnes la Coquette, France) according to National committee for Clinical laboratory system (NCCLS) [13] by using disks from BBL Microbiology system. The tested antibiotics were cefotaxime, ceftazidime (30µg), gentamicin (10µg), Amikacin (30µg), kanamycin (30µg), Imipenem, Trimetroprim Sulfamethoxazole (1.25/23.75µg), Chloramphenicol (30µg), Minocyclin (30µg) and Ciprofloxacin (5µg).

Biotyping of isolates

The biotyping of isolated strains were performed as previously described by Grimont *et al.* [14].

Statistical analyses

Multivariate analysis was performed to demonstrate associations between individual variables and organism acquisition, Two-tailed *p* values of <0.05 were considered statistically significant. All statistical analyses were performed using SPSS software (SPSS 10.0 for Windows).

Results:

Prevalence of S. marcescens among patients

A total of 790 children were enrolled in this study and 526 (66.5%) were males and 264 (33.5%) females.

The mean age was 24.9 months. The mean weight was 11.5 kg (range 6.1-19.0, 95% CI [11.3-11.7]) and the mean height 83.4 cm (range 59-110, 95% CI [82.7-84.2].

The prevalence of nosocomial infection was 41% (324/790) during the period of the study. A total of 123 *S. marcescens* non duplicate isolates were recovered from a variety of clinical specimens corresponding to 38% of the cases of nosocomial infections. The organisms were most commonly isolated from blood specimens 65 (52.8%) followed by urinary tract infections 31 (25%), pneumonia 12 (9.7%) and others infections15 (12%). *S. marcescens* was isolated in pure culture in 100% of cases. Only 10% of the isolates are producer of the prodigiosin.

Isolation of S. marcescens in the hospital environment

The Table1 present the distribution of the isolates from various samples. A total of 93 samples from the hands of nursing staff and 940 samples from environmental sources and medical devices were screened for *S. marcescens*. The hand specimens yielded 31 isolates. A total of 108 isolates were obtained from various environmental samples and medical devices. Two strains were recovered from the antiseptic solution. The overall isolation rate of

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S. marcescens in the hospital environment was 11.5%.

Table 1: Distribution per sampling sites of 108 Serratia marcescens strains isolated from the hospital environment and staffs hand sample site 31 isolates

Environment site 940 samples	Positive number108 (11.5%)
Floors	22 (23,6)
Locker	2 (2.1)
Mobile instrument table	6 (6.5)
Bed frame	12 (12.9)
Saline irrigation solution	6 (6.5)
Liquidantiseptic	10 (10.7)
Over-bed table	6 (6.5)
Remedy bottle	10 (10.7)
Light switch.	9 (9.7)
Table at. nursing room	10 (10.7)
Personnel hand culture 93 samples	Positive number 31 (33%)
Nurses (20)	(12/20) 60%
Physician(5)	(2/5) 40%
Others (6)	1 (1/6) 16,6%

Biotyping of S. marcescens isolates.

Biotyping of 262 isolated strains of *S. marcescens* from all origin showed four (4) distinct biotypes: A5, A8b, TCT and A1. One predominant biotype A5 is present in 75 patients and represents 90% of isolates from the hand of nursing staff (table 3). This type was resistant to all antibiotics tested, except imipenem and ceftazidime.

Table 3 : Antimicrobial susceptibility patterns of *Serratia marcescens* isolates associated to biotype

		Antimicrobial susceptibility pattern									
Biotype	Number	CEF	CTX	GM	AMK	KA	IMI	CIP	SXT	Mino	CHL
A5	148	S	R	R	R	R	S	S	R	R	R
A8b	43	R	S	R	S	S	S	S	R	S	S
TCT	30	S	S	S	R	S	R	R	R	S	R
A1	41	S	S	S	R	S	S	S	S	S	S
TOTAL	262	16.4	56.4	73	83.5	56.4	11.4	11.4	84.3	56.4	68

CEF, ceftazidime, CTX, cefotaxim GM, Gentamicin; KA, Kanamycin; AMK amikacin ; SXT, Trimetroprimsulfamethozolin; CIP ciprofloxacin, CFT; Minominocycline, CHL, Chloramphenicol

Antimicrobial susceptibility patterns of Serratia marcescens isolates related to biotype

Table 2 shows the susceptibilities to 10 antibiotics of the 262 isolates *S. marcescens*. Fifty six percent (56%) of the isolates was simultaneously resistant to amikacin, minocyclin, Trimetroprim-Sulfamethoxazole, kanamycin Cefotaxim and chloramphenicol. The susceptibility and resistance profile of all isolates in this study have shown that Imipenem, ciprofloxacin and ceftazidime possess the higher efficacy while Trimethoprim sulfamethoxazole and amikacin possess lower efficacy. The biotypes A5, A8b, TCT are usually recovered from hospital environment.

Table 2: Biotyping of *Serratia marcescens* from Jun to December 2009 at pediatric unit in Benin

Bio groups	Clinical isolates 123	Environmental isolates 108	Hand isolates 31	Total 262
A5	75 (61%)	45 (41.6%)	28 (90%)	148 (56.4%)
A8b	23(19%)	19 (17.5%)	1 (3.2%)	43(16%)
TCT	20 (16%)	8 (7.4%)	2(6.4%)	30 (11.4%)
A1	5 (4%)	36 (33%)	0	41 (15.6%)

Intervention and termination of the outbreak

Investigation of care practices in the all hospital revealed several flaws. After the procedural review and survey, antiseptic solution was suspected to be the probable source of the outbreak. Twenty (20) samples from the same batch file of this antiseptic and from unopened boxes available as a stock in the hospital store were analyzed. Quantitative culture of the contaminated antiseptic revealed a mean of 178100>10^5 CFU/mL (51200 195000 CFU/mL). Samples taken from other batch file were all negative for *S.marcescens*. The all distributed antiseptic solution was recalled immediately, adherence to hand antisepsis was encouraged in January 2010. Standard precautions were reinforced by repeated educational sessions. Incriminated antiseptic was produced by a local factory, packed in 5 L plastic bottle. In the hospital this antiseptic is dilute with sterile water and reconditioned in 500 mL plastic bottle by the nurse for use.

Discussion

S.marcescens was considered to be non-pathogenic until recent decade when they were shown to be important causes of infections in patients with underlying diseases[15,16]. Increasing reports in the literature document the existing of nosocomial transmission of *S marcescens*[9,10]. The identification of a cluster of 12 children with normal immunity, infected with *S. marcescens* in May 2009 prompted this investigation. It revealed

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the first documented outbreak of *S. marcescens* in pediatric unit of CHDZ/C hospital.

The prevalence of nosocomial *S. marcescens* was 38% in our study. This prevalence was higher than 2% reported by Ostrowsky *et al.*[17], but comparisons are problematic due to different methods of surveillance and heterogeneous resident populations.

Remarkable in the present study was the results of multivariate analysis of factors associated with infections/colonization with this bacterium (Table 4). The use of invasive devices was an important risk factor in our analysis. Several factors may explain the cross transmission such as duration of hospitalization was strongly associated with nosocomial acquisition of multidrug resistant *Serratia marcescens* (Table 4).

Table 4: Multivariate analysis of factors promoting colonization and infection with Serratia marcescens in pediatric unit in Benin

Variable	OR	95% CI	p-value
Disruption of bowel flora	0.90	0.30 2.60	
Surgery or instrumentation of the urinary tract.	2.80	1.40 5.83	0.0035
Duration of hospitalization	3.17	1.63-6.16	0.0003
Prolonged antibiotic therapy	3.50	1.94-6.32	0.0007
Invasive devices	8.69	3.82-19.51	0.0002

Most clinical isolates of *S. marcescens* reported previously were resistant or even multiresistant to antibiotics [2,10]; about 56% of the *S. marcescens* strains isolated in this study were resistant to more than five antimicrobials and was comparable to the mean of 53 reported by Manikandan *et al.*[10].This reflected the fact that, Trimethoprim sulfamethoxazole and gentamycin were the most commonly prescribed antibiotics in the hospital even before the results of analyses and also the most easily available in the market without prescription and because they were also very cheap in terms of cost.

The present study confirms that bacterial resistance would be a greatest problem in developing countries. The growth of *S.marcescens* in the environment has been investigated in relation to water, disinfectants and plastics such as blood bags[4,18] and corroborated our result. Although hospital staff and patients are considered the most important source of nosocomial microorganisms, there is growing evidence that the colonized hospital environment is also of substantial importance[19,20]. The high population density of *S.marcescens* in the environment (11.5%) especially in diluted antiseptic solution placed on sink may partly explain the presence of this organism on the hands of the nursing staff, because after hand washing they used contaminated antiseptic solution. The single sink in the room was not adequate for effective hand-washing practice; and there was consequently insufficient use of a hand-sanitizer (70% ethyl alcohol, water plus glycerin) to disinfect the hands. The role of the environment in nosocomial spread of *S. marcescens* has been established[4,8]. It seems likely that transient carriage on the hands of the healthcare personnel was the main path of spread of this bacterium. The biotyping and susceptibility typing of antimicrobial agents tested presented a good correlation. The presence of 4 types with one dominant in our sample, suggests an outbreak with multidrug resistant *S. marcescens* which was paralleled by a small cluster of *S. marcescens* cases caused by an endemic, nosocomial strain. Three of the four biotype identified in his study are mainly encountered in the hospital, and are frequently multiresistant to drug. Nosocomial outbreaks of *S. marcescens* are commonly associated with multiple antimicrobial resistances since *S. marcescens* have inducible chromosomal beta-lactamases and R-factors which encode genes for particular drug resistance according to Tara *et al.* [21, 22].

The presence of this outbreak was unnoticed as it was masked by endemic incidence of ordinary *S. marcescens* infections. During this time care practices in the paediatrics' unit had several inherent flaws, Hand hygiene compliance was poor. All these situations are known to promote colonization of hospital environments by organism[2, 23].

The strict infection control measure most definitively contributes to the significant reduction of the bacterium in this sector of the hospital.

Conclusion:

This outbreak revealed that *S. marcescens* can easily spread within the healthcare setting and offers a sobering reminder of the need to maintain high standards of hygiene. Biotyping analysis in our context helped to elucidate the epidemiological implications of this multiresistant *S. marcescens* outbreak, which coincided with a cluster of ordinary unrelated endemic *S. marcescens*. Traditional control measures

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terminated the pediatric unit outbreak. Healthcare associated transmission of strains raises important issues for infection control.

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Disclosure statement

Authors have nothing to disclose

Ethical clearance:

Was obtained from the Ministry of Public health of Benin Republic under protocol number:- 2959/MSP/DC/SG/DRH/SPREA-05-2002. But it was important to notice that, the strains were de-identified and analyzed anonymously and the strains, not a human, were studied.

References:

1. Friedman ND, Kotsanas D, Brett J, Billah B, Korman TM: Investigation of an outbreak of *Serratia marcescens* in a neonatal unit via a case-control study and molecular typing. Am J Infect Control 2008; 36 (1):22-8.
2. Maragakis LL, Winkler A, Tucker MG, Cosgrove SE, Ross T, Lawson E, Carroll KC, Perl TM: Outbreak of multidrug-resistant *Serratia marcescens* infection in a neonatal intense care unit. Infect Control Hosp Epidemiol 2008; 29 (5):418-23.
3. Yannelli B, Schoch PE, Cunha BA: Serratia infections in the hospital. Clin Microbiol. Newsl 1987; 9:157-60.
4. Schaberg DR, Alford RH, Anderson R, Farmer JJ 3rd, Melly MA, Schaffner W: An outbreak of nosocomial infection due to multiply resistant *Serratia marcescens*: evidence of interhospital spread. J Infect Dis 1976; 134(2):181-8.
5. Metintas S, Akgun Y, Durmaz G, Kalyoncu C: Prevalence and characteristics of nosocomial infections in a Turkish university hospital. Am J Infect Control 2004; 32 (7):409-13.
6. Bosi C, Davin-Regli A, Charrel R, Rocca B, Monnet D, Bollet C: *Serratia marcescens* nosocomial outbreak due to contamination of hexetidine solution. J Hosp Infect 1996; 33(3):217-24.
7. David MD, Weller TM, Lambert P, Fraise AP: An outbreak of *Serratia marcescens* on the neonatal unit: a tale of two clones. J Hosp Infect 2006; 63(1):27-33.
8. Royo P, del Valle O, Boquete T: Epidemiology of *Serratia marcescens* between 1987 and 1995 at Vall d'Hebron Hospital. Enferm Infecc Microbiol Clin 1997; 15 (10):519-27.
9. Su LH, Ou JT, Leu HS, Chiang PC, Chiu YP, Chia JH, Kuo AJ, Chiu CH, Chu C, Wu TL, Sun CF, Riley TV, Chang BJ; Infection Control Group: Extended Epidemic of Nosocomial Urinary Tract Infections Caused by *Serratia marcescens*. J Clin Microbiol 2003; 41(10): 4726-32.
10. Manikandan S, Ganesapandian S, Manoj Singh, Kumaraguru AK: Antimicrobial Susceptibility Pattern of Urinary Tract Infection Causing Human Pathogenic Bacteria. Asian J Med Sci 2011; 3(2): 56-60.
11. Blot S, Vandewoude K, Blot K, Colardyn F: Prevalence and risk factors for colonisation with Gram-negative bacteria in an intensive care unit. Acta Clin Belg 2000; 55(5): 249-56.
12. Freney J, Renaud F, Hansen W, Bollet C : Précis de Bactériologie Clinique. Eds ESKA Paris, 2000.
13. NCCLS (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial disk susceptibility tests. Approved standard, 7thed. NCCLS document M2-A7 NCCLS, Wayne (PA), The committee, 2000.
14. Grimont PA, Grimont F: Bioting of *Serratia marcescens* and its use in epidemiological studies. J Clin Microbiol 1978; 8(1): 73-83.
15. Woodward HMM, Clark KB: A case of infection in man by the bacterium prodigiosum. Lancet 1913; 1:314-315.
16. Wheat RP, Zuckerman A, Rantz LA: Infection due to chromobacteria: report of eleven cases. AMA Arch Intern Med 1951; 88(4): 461-6.
17. Ostrowsky BE, Whitener C, Bredenberg HK, Carson LA, Holt S, Hutwagner L, Arduino MJ, Jarvis WR: *Serratia marcescens* bacteremia traced to an infused narcotic. N Engl J Med 2002; 346(20): 1529-37.
18. Yah SC, Eghafona NO, Forbi JC: Plasmid-borne antibiotics resistant markers of *Serratia marcescens*: an increased prevalence in HIV/AIDS patients. Sci Res Essays 2008; 3 (1):28-34.
19. Dancer SJ: Mopping up hospital infection. J Hosp Infect 1999; 43(2):85-100.
20. Talon D: The role of the hospital environment in the epidemiology of multi-resistant bacteria. J Hosp Infect 1999; 43(1):13-7.
21. Echols RM, Palmer DL, King RM, Long GW: Multidrug-resistant *Serratia marcescens* bacteriuria related to urologic instrumentation. South Med J 1984; 77(2): 173-7.
22. Matsuo T, Chen J, Minato Y, Ogawa W, Mizushima T, Kuroda T, Tsuchiya T: SmdAB, a Heterodimeric ABC-Type Multidrug Efflux Pump, in *Serratia marcescens*. J Bacteriol 2008; 190(2):648-54.
23. Oie S, Hosokawa I, Kamiya A: Contamination of room door handles by methicillin-sensitive/methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 2002; 51(2):140-3.

Competing interests

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