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Epidemiological Distribution of *Serratia marcescens* from Environment and Human Subjects in Ile Ife South Western Nigeria: An Update

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Abstract

This study investigated the epidemiological distribution of *Serratia marcescens* among hospitalized individuals, hospital environments and halls of residence of Obafemi Awolowo University in Ile Ife, Osun state. Two hundred and twenty samples from clinical and non-clinical sources were collected with ethical clearance approval (ERC/2018/09/02) from the Obafemi Awolowo University Teaching Hospital Complex, (OAUTHC) Ile-Ife, Osun State advisory committee. They were cultured on sorbitol Mcconkey agar infused with 200 u/ml of colistin for the selective isolation of *serratia* species. Non-duplicate colony was picked from each cultures and characterized biochemically with the use of microbat 24 E kit to identify various species isolated. Thirteen (61.9%) *serratia marcescens* out of the 21 (9.55%) *Serratia sp.* isolates were recovered from the study area. Other species isolated were *S. fonticola* (4.8%), *S. Rubidae* (4.8%) and *serratia liquefaciens* complex (28.6%) serratia species were significantly associated with the community (OAU) than the hospital (OAUTHC) ($p<0.001$). Clinical research should be targeted on alternative pathogens control techniques as quorum quenching. Communities also requires enhanced capabilities for the detection of possible resistance mechanisms which must be adapted by private as well as public laboratories to implement appropriate infection control practices, and to prescribe effective chemotherapeutic agents against pathogenic strains of *S. Marcescens* in the study area.

Keywords: *Serratia marcescens*; Liquefaciens; Oxoid; Dissolved colistin; Polymyxin b powder

Introduction

Serratia marcescens accounts for most infections caused by members of the *Serratia* genus and have been recovered from various clinical specimens. They have characteristic pink-red pigmentation of prodigiosin. This is commonly observed in certain strains of *Serratia marcescens*, *S. plymuthica* and *S. Rubidaea*. Hospital isolates *Serratia marcescens* are rarely pigmented, and both forms have been isolated from infections and outbreaks within the community and hospital settings [1]. The fast transmission of *S. marcescens* in hospital environments among immunocompromised individuals, revealed that 65% of *serratia* infections in Canada were transmitted in the community. A typical example was community acquired *S. Marcescens* ocular infection transmitted even among immunocompetent hosts. The organism was a contaminant in the milk room with breast pumps and feeding bottles as fomites for transmission of the pathogen [2]. Their report justified earlier claims that milk makes a good medium for proliferation of *serratia marcescens*. Their ability to thrive at low pH may account for evasion of gastric acid in stomachs of the neonates after oral ingestion of pathogens. Attributed low *serratia* outbreaks reports in African countries to poor surveillance [3]. They opined that poor hygiene practices, low awareness of hospital-acquired infections among health workers, malnutrition, misuse of antibiotics characterize many developing countries and heighten the chance of *serratia* nosocomial and community-acquired infections. They reported an outbreak of *S. Liquefaciens* nosocomial infections with 3 deaths out of 44 children infected in Gambia. In South attributed 20% of 30 total outbreaks reports in neonatal units from 2005-2015 to *Serratia marcescens*. Also, Gonzalez-attributed high probability of outbreaks in South Africa hospital environments to overcrowding, understaffing and excess equipment sharing and low reports to lack of facilities for outbreaks investigation [4]. Reported that the major routes of transmission of *Serratia marcescens* are ingestion of contaminated food or drinks, fomite-contact as in case of hospital workers and surgical equipment.

Fomites and hospital equipment are particularly important in transmission, as *S. marcescens* has earned resistance record to common disinfectants and sterilization procedures. Reported a multistate outbreak of *Serratia marcescens* bloodstream infections involving California (Los Angeles), New York, North Carolina, Massachusetts, Georgia (Atlanta) and New Jersey, they implicated magnesium sulphate as the reservoir acting as source of *S. Marcescens* transmission which had initially contaminated the solution circulated across the states by a national supplier [5]. The outbreak was successfully contained after the CDC called off the incriminating batch of the intravenous fluid in 2008. Also reported a similar outbreak in Chicago, Illionois in about the same time with. In France, an outbreak of *Serratia marcescens* in Neonatal Intensive Care Units (NICU) due to contaminated hospital equipment in 2008 was reported by Buffet. As at the time of this study, there are no published reports of *Serratia marcescens* outbreak in Nigeria although

several study had successfully incriminated the pathogen in nosocomial and community-acquired infections. Overcrowding, understaffing, excessive sharing of equipment among patients implicated elsewhere as predisposing factors to spread of hospital-acquired infections and outbreak were observed in the sampled wards in course of this study. The study seek to isolate and identify *Serratia marcescens* base on frequency of occurrence in clinical and non-clinical sources in Ile-Ife and to determine the percentage distribution of the organism from various sources among subjects and the environment [6].

Materials and Methods

Samples collection

A total of 220 samples were collected from hospitalized patients of Obafemi Awolowo university teaching hospitals, hospital and hall environments of Obafemi Awolowo university within October 1 to December 30, 2018 with an approval (ERC/2018/09/02) by the medical advisory committee. Samples collected were urine from catheterized patients, swabs of surfaces, door handles, sinks and soap containers from selected wards. Swabbed samples of the university hall environments including bathroom tiles, and door handles were also collected following standard microbiological methods [7]. Urine samples were collected in sterile universal bottle and transported to the laboratory. Each swabbed sample were transported in bijou bottles containing 5 ml of sterile tryptic soy broth. All samples were transferred to the laboratory for immediate cultures on freshly prepared sorbitol MacConkey agar infused with 200 U/ml of colistin [8].

Isolation of bacteria

All samples were inoculated by spread plate method on Sorbitol MacConkey Agar (SMAC; Oxoid CM0813; Hampshire, England) infused with Polymyxin B (colistin) (200 U/ml) as described by with slight modification. Following manufacturer's recommendation, sorbitol MacConkey agar powder were dissolved in sterile distilled water (5.15 g/100 ml) in an air-tight conical flask. The mixture was homogenized and autoclaved at 121°C for 15 minutes. The molten agar was allowed to cool to 45°C prior to addition of dissolved colistin. Vials of Polymyxin B powder (Alvogen; New Jersey, USA) 500,000 U were dissolved in sterile distilled water and added to sterile, molten but cooled SMAC to make 200 U/ml concentration [9]. Each conical flask was shaken to ensure even distribution of colistin in the molten agar, and poured into sterile petri dishes. The agar plates were allowed to gel, and dried in laboratory oven (45°C) to remove condensed water vapors. Each sample was inoculated by spread plate method. The inoculated agar plates were incubated at 28°C ± 2°C for 24 hours to enhance pigmentation of *serratia sp.* A colony of pink/red sorbitol/non-fermenting pigmented or non-pigmented colony were picked per plate. Pure colonies of each isolate was obtained by sub culturing each isolate on freshly prepared nutrient agar prior to biochemical characterization for identification [10].

Preliminary biochemical characterization of isolates

Cultural, biochemical characterization and Identification of *serratia sp.* were carried out following procedures of standard procedures on Gram staining, motility test, oxidase test, gelatin hydrolytic test, DNA se and Casein hydrolysis were conducted [11].

Biochemical tests (microbact 24E identi ication kit)

Further identification of isolates was done using Microbact 24E identification (Basingstoke, England) which is a standardized micro-substrate system for the identification of *Enterobacteriaceae* and common miscellaneous Gram-negative *bacilli*. Each kit of 24 miniature biochemical tests identifies GNB to species with proven degree of accuracy. Organism identification is based on pH change and substrate utilization [12,13]. Each well was inoculated with an isolate suspension that reconstitutes the media. During incubation at 37°C for 24 hours and 48 hours, metabolic reactions produced colour changes that were either spontaneous or revealed by the addition of reagents. The results were recorded on report forms and interpreted using the Microbact identification Package. *E. coli* ATCC 25922 served as the control strain [14].

Preparation of inoculum and inoculation

Two to three isolated colonies from the 18 hours-24 hours cultures were emulsified in 5 mls of normal saline; it was mixed thoroughly to prepare a homogenous suspension. The microbact test kit used was 24E. With the aid of sterile micro pipette and sterile tips 100 µl of the homogenous suspension were added to each test strips [15]. The wells lysine, ornithine, hydrogen sulphide, containing the homogenous suspension were overlaid with mineral oil. The strips were incubated for 24 hours and 48 hours at 37^{O}C. The results were read after adding necessary reagents such as indole, VP and TDA.

Nitrate reduction test

This test was performed in well 7 (ONPG) after reading the ONPG reaction. One drop of nitrate reagent A and 1 drop of Nitrate Reagent B were added to the well [16].

Reading of test strips

The 24E strip was read after 24 hours and the reactions were evaluated as "positive" or "negative" by comparing to the colour chart and recording the results. The following reagents were added; well 8 (Indole production), 2 drops of indole (Kovacs) reagent was added and was evaluated within 2 minutes of the addition of the reagents. Well 10 (Voges-Proskauer-reaction), one drop each of VPI and VPII reagent was added and evaluation was done 15 minutes-30 minutes after the addition of the reagents. Well 12 (Tryptophan deaminase), one drop of TDA reagent was added and the test was evaluated immediately after the addition of the reagent [17].

Interpretation of results

An octal coding system was adopted for Microbact. Each group of three reactions produces a single digit of the code. Each digit of the code is formed by adding the indices of the positive reactions. A total of eight digits from the eight groups form each code number (octal code). These codes were entered into the software package from microbact. Percentage probabilities were recorded along with isolate IDs.

Analysis of data

Data obtained were analyzed and presented as frequencies and percentages. Data were compared with the use of the two tailed fisher's exact test with the SPSS statistical program (version 22). All reported p-values were two-sided and a p-value of less than or equal to 0.05 was considered to be statistically significant (**Table 1**).

Results and Discussion

Incidence of*serratia* species from the study

The results showed 9.55% prevalence of *Serratia* isolates in the study population where two hundred and twenty (220) non-duplicate consecutive samples were collected from hospitalized patients Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), hospital environments and hall environment of the University (**Table 2**). *Serratia* species are significantly associated with the community (OAU) than the hospital (p<0.001).

The *serratia* species were isolated from the diverse range of specimens between October and December 2018. All 21 (100%) *serratia* species were isolated from the environment and none (0%) from humans. The organisms recovered are *serratia marcescens* 13 (61.9%), *serratia fonticola* 1 (4.8%), *serratia rubidaea* 1 (4.8%) and other *serratia sp.* 6 (28.6%) as presented in **Figure 1**. These isolates were cultured from the environment and fomites from Obafemi Awolowo University (OAU) and Obafemi Awolowo University Teaching Hospital complex (OAUTHC), Ile-Ife; OAUTHC renal ward sinks (n=2) and OAU hall of residences' environments (n=19). *Serratia* species are significantly associated to the community (<0.001) as is represented in **(Table 1)**.

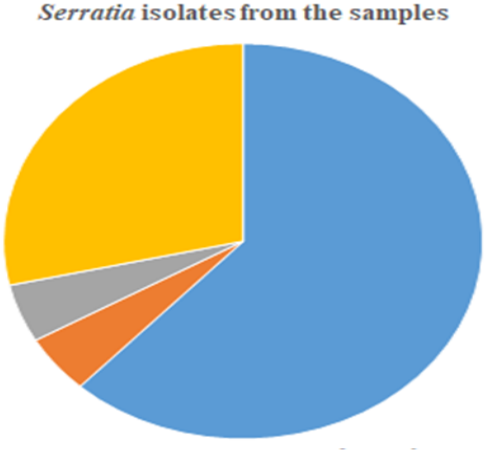

Figure 1: *Serratia* species distribution. Note: *S. marcescens*; *S. fonticola;* *S. rubidaea;* *Other Serratia spp.*

Table 1: *Serratia* species distribution.

Well no.	Designation	Reaction principle	Reaction colours	
			Negative	Positive
1	Lysine	Lysine decarboxylase	Yellow	Blue-green
2	Ornithine	Ornithine decarboxylase	Yellow-green	Blue
3	H_2S	H_2S production	Straw colour	Black
4	Glucose	Glucose fermentation	Blue-green	Yellow
5	Mannitol	Mannitol fermentation	Blue-green	Yellow
6	Xylose	Xylose fermentation	Blue-green	Yellow
7	ONPG	Hydrolysis of o-nitrophenyl-ß-d-galactopyranoside (ONPG) by action of ß-galactosidase	Colourless	Yellow
8	Indole	Indole production from tryptophan	Colourless	Pink-red
9	Urease	Urea hydrolysis	Straw colour	Pink-red

10	VP	Acetoin production (Voges-Proskauer reaction)	Straw colour	Pink-red
11	Citrate	Citrate utilization (citrate is the only source of carbon)	Green	Blue
12	TDA	Production of indolepyruvate by deamination of tryptophan	Straw colour	Cherry red
13	Gelatin	Gelatin liquefaction	Colourless	Black
14	Malonate	Malonate inhibition	Green	Blue
15	Inositol	Inositol fermentation	Blue-green	Yellow
16	Sorbitol	Sorbitol fermentation	Blue-green	Yellow
17	Rhamnose	Rhamnose fermentation	Blue-green	Yellow
18	Sucrose	Sucrose fermentation	Blue-green	Yellow
19	Lactose	Lactose fermentation	Blue-green	Yellow
20	Arabinose	Arabinose fermentation	Blue-green	Yellow
21	Adonitol	Adonitol fermentation	Blue-green	Yellow
22	Raffinose	Raffinose fermentation	Blue-green	Yellow
23	Salicin	Salicin fermentation	Blue-green	Yellow
24	Arginine	Arginine dihydrolase	-	-
-	-	24 hours	Yellow	Green-blue
		48 hours	Yellow-green	Blue

Table 2: Isolated *Serratia sp*. and their sources.

Isolates designation	Source	Presumptive identity
RLW1	Renal ward sink 1	*S. marcescens*
RLW4	Renal ward sink 4	*S. fonticola*
DH5	Swab of door handle	*S. marcescens*
H1.1	Swab of bathroom tiles of PG hall H	*S. rubidaea*
H3.4	Swab of bathroom tiles of PG hall H	*Serratia* complex
F2.3	Swab of bathroom tiles of PG hall F	*Serratia* complex
J1.4	Swab of bathroom tiles of PG hall J	*Serratia* complex
F3.3	Swab of bathroom tiles of PG hall F	*Serratia* complex

F3.4	Swab of bathroom tiles of PG hall F	*Serratia* complex
I2.1	Swab of bathroom tiles of PG hall I	*S. marcescens*
E9	Swab of bathroom tiles of PG hall E	*S. marcescens*
B1	Swab of bathroom tiles of PG hall B	*S. marcescens*
W3	Mozambique hall	*S. marcescens*
A5	Door handle of PG hall A	*Serratia* complex
ANG3	Anglomos hall door handle	*S. marcescens*
W5	Alumni hall	*S. marcescens*
MOR2	Swab of bathroom tiles of Moremi hall	*S. marcescens*
ANG2	Door handle swab of anglomos	*S. marcescens*
MOZ2	Swab of bathroom tiles of Mozambique hall	*S. marcescens*
MOZ4	Swab of door handle of Mozambique hall	*S. marcescens*
PG4	Water tap handle of PG hall	*S. marcescens*

Table 3: Distribution of *serratia* species from the study area.

Organism	**Sample source N=220**		**FE (p-value)**
OAU halls of residence n=167 (%)	OAUTHC n=53 (%)		
S. *marcescens*	12 (7.19)	1 (1.89)	-
S. *fonticola*	0 (0.00)	1(1.89)	
S. *rubidaea*	1 (0.60)	0 (0.00)	-
Others	6 (3.59)	0 (0.00)	-
Total (%)	19 (11.38)	2 (3.77)	24.381 (<0.001)

Serratia species are opportunistic pathogens, classified as members of the *Enterobacteriaceae* family, innocuous in the environment and a rare cause of human disease but are now well-established as a cause of both nosocomial and community infections for the past four decades. There have been reports regarding the identification, antibiotic susceptibility, pathogenicity and epidemiological investigation of this microorganism. The outbreaks of *serratia* species have been described; and reservoirs of the pathogens identified in a variety of clinical settings and different environmental sources that makes accurate identification of *serratia* species important in defining outbreaks. The occurrence of *serratia* species in the study area is 9.5%. The numbers of isolates recovered from the community were higher (90.5%) than those obtained from the hospital (9.5%). *serratia marcescens* was the most frequently isolated organism (13/21; 61.9%), followed by *Serratia rubidaea* (1/21; 4.7%), *serratia rubidaea* (1/21; 4.7%) other *Serratia* species were 28.6%. This study showed 4.8% (1/21) prevalence of *Serratia marcescens* from the hospital; this result is a bit lower that reported a prevalence of 5% and 7.7% respectively. All (100%) the *Serratia spp*. identified and characterized in this study were from the environment and none (0%) from human unlike that reported lower environmental occurrence.

Conclusion

Strategic implementation in preventing hospital-acquired infections depends on awareness of reservoirs of pathogens, routes of transmission and epidemiology of the microorganisms. Therefore, we recommend prompt diagnosis of the colonized human subjects and the environment couple with appropriate

implementation of infection control measures which are critical factors to limiting the spread of *S. marcescens*. Steady surveillance as an integral part of infection control programs should be adequately maintained in the study area.

Author Contributions

ABO planned and performed the experiment while JOA Supervised and Coordinated all experiment and wrote the manuscript with contributions from both Authors.

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