

## Enhancing Contact Tracing for *Serratia marcescens* Biofilm on High-Usage Body Towels in Rivers State Bathrooms

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### Abstract

The placement of body towels before and after use in bathrooms where *Serratia marcescens* proliferates calls for concern, as *Serratia marcescens*, an airborne opportunistic pathogen has been reported keratinolytic. *Serratia marcescens*, a bacterium commonly noticed with a pink or red slimy appearance in toilet sinks, bowls, and tiles presents an aesthetically, unappealing and disgusting appearance in the toilet surroundings. The study aimed to trace the presence of *Serratia marcescens* on frequently used body towels hung in the bathroom doors. Swabs from forty (40) differently used towels were collected from twenty (20) volunteered homes and analyzed using standard microbiological procedures. Microbiological procedures involved inoculating the swab sample on a prepared peptone broth and plating on MacConkey agar media, followed by identification and streak of the recovered isolate onto Congo red agar media for biofilm formation. Results showed the recovery of *Serratia marcescens* isolates. The three homes showed a *Serratia marcescens* count of  $3 \times 10^1$ ,  $1 \times 10^2$ , and  $7 \times 10^1$  CFU per swab for house units C, I, and P respectively. *Serratia marcescens* could form a biofilm, a basic feature that allowed it to thrive on a body towel. The results derived strongly identified the presence of *Serratia marcescens* biofilm on body towels hung in the bathrooms. This could have health implications for towel users due to the bacteria's keratolytic properties. Hence, the need for constant surveillance to support effective measures of hygiene, aimed at preventing the spread of *Serratia marcescens* is recommended.

**Keywords** *Serratia marcescens*, Body Towels, Biofilm

### INTRODUCTION

*Serratia marcescens* is an opportunistic, nonpathogenic, saprophytic bacterium that was first discovered by Bizio, an Italian pharmacist in 1819. It is found in the environment, mostly in medical equipment, lotions, and sinks where it is implicated as a source of epidemics. As an epidemic form, it is associated with urinary tract infections, surgical wounds, respiratory tract, and soft tissues (Nazzaro, 2019). *Serratia marcescens* expresses prodigiosin, a pigment responsible for its red color, and a beta-lactamase, a gene that confers antibiotic resistance (Khanna et al., 2013). *Serratia marcescens* is commonly, noticed with a pink or red-colored slimy appearance seen in toilet bowls, sinks, tiles and a shower of bathrooms (Sinclair & Gerba, 2010). It is reported to show a poor aesthetic view of the toilet bowls, sink, and tiles (Sinclair & Gerba, 2010). The challenge thrown-off, gives the bathroom a dirty appearance, as the bathroom appearance is aesthetically unappealing and disgusting in some circumstances. The bathroom, which is a room in a building, is comprised of a tap, shower, toilet bowl, and most times a washbasin. The bathroom is somewhat regarded as an open space in the house, although architects have stepped further to make or create it luxurious (Corradi et al., 2020). The bathroom is severally challenged by the evasion of *Serratia marcescens* due to its damp nature (Jayarajah et al., 2019). According to Abney et al. (2011), the bathroom is bereft of proper ventilation and damp condition. *Serratia marcescens* is mostly observed and reported to be associated with bathrooms with specific areas of the water system, the gut of tiles, the toilet bowl along the water line, and at the openings where the water enters the

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toilet bowl (Sinclair & Gerba. 2010). Infectious agents such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella* sp. etcetera in the bathroom have been previously associated with disease outbreaks due to improper hygiene practices of cleaning and disinfection of sanitary appliances (Matini et al., 2020). Enteric pathogens such as *Escherichia coli* are found in large numbers after every use by users via defecation (Matini et al., 2020). Feces containing the *Escherichia coli* contaminate other areas of the toilet such as the bowl sides and rim and thereafter, during flushing the microbes disperse to other areas of the bathroom. Flushing of toilets releases microbes into the air (Sinclair & Gerba. 2010). Sinclair and Gerba (2010) observed that bacteria attached to the sidewalls of toilet bowls and rims contribute to the formation of bacteria aerosols. Their study also noted that closing the toilet lid never prevented the release of bacteria into the air. Thus, the aerosolization of *Serratia marcescens* (Salarizadeh et al., 2016) may lead to the colonization of surfaces. *Serratia marcescens* in particular has the unique property of being airborne (Khanna et al., 2013). The frequent placement of body towels in the bathroom could expose the towels to bacteria. According to Kim et al. (2019), the soft piece of cloth (towel) is used to dry the body after bathe and is always placed on the doorknob after every use where it dries from its damp state. Body towels used in the home share a common purpose of being moisture-absorbent. In this process, the towel gets dirty easily and remains moist with microbes embedded (Kim et al., 2019). Kim et al. (2019) reported that users of body towels do not wash their towels often and these towels are largely contaminated with an average *Escherichia coli* count of  $1.64 \times 10^5$  bacteria per square inch. The study, therefore, aimed at tracing *Serratia marcescens* biofilm on frequently used body towels found in some bathrooms in Rivers State, Nigeria.

## LITERATURE REVIEW

Studies conducted by Khanna et al. (2013) tried to find out what caused the pink-colored fungi (mold) in the bathroom and they found that it was not a fungus (mold) but a bacterium. They further reported that the colored substance was not from the colored soap in the bathroom as speculated, but from *Serratia marcescens* (Khanna et al., 2013). Consequently, the bacterium is soap-loving (Stewart et al., 2012), and reported to initiate growth continuously, in the existence of the constant wet (water) surface the bathroom offers and in addition to the phosphorous and fatty residue present in the soap (Stewart et al., 2012). Khanna et al. (2013) reported that *Serratia marcescens* does not pose any health challenge even after being contracted or touched as it is pathogenic to an immunocompromised person when transmitted or ingested or entry through open wounds where severe complications and infections arise. Crowdy et al. (1984) noted the likelihood or probability of microbial infections caused by aerosol contamination of surfaces after flushing a toilet. Following these, Sinclair and Gerba (2010), were able to show aerosol generation in public restrooms specifically, toilets with little or no ventilation frequency. These observations lead to health assessment in terms of hygiene and sewage management to eliminate disease (Crowdy et al. 1984). Used towels harbor a large density of diverse microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Candida albican*, etc), and thus spreads these microorganisms to users which in turn infect the body and cause diverse diseases such as skin infections which include warts, jock itch, athlete's foot, and toenail fungus. Following this disease spread, the importance of personal hygiene cannot be overemphasized with special reference to towel handling. Thus, a survey carried out by Mishra and Babel (2020) to determine the frequency of how towels are been washed by users. Respondents report showed that 30, 10, 40, and 20 percent of the respondent washed their towel once a month, after every use, once a week, and twice a week respectively. This report, therefore, presented users with a template for assessing towel handling hygiene. In the same survey, the respondents were asked for the type of detergent used to wash their towels.

Mishra and Babel's (2020) additional report, revealed that 20, 30, 10, 20, and 30 percent used soap, detergent, liquid detergent, soap powder, and detergent powder respectively to wash their towels. This unconsciously, reflected that the respondents do not have good knowledge of how to handle their towels (Abdul & Hassan, 2012). In line with the reported observations, the formation of biofilm, a complex microbial system formed by several microbial (bacteria and fungi) species cannot be overlooked as bacteria biofilm gets glued to these towels. The attachment of bacteria biofilm to the substratum (towel) is unique (Stewart et al., 2012). Although, several bacteria have been reported to form biofilm in tap water, amongst which; *Listeria monocytogenes* and *Salmonella* species are implicated. The ability of the bacteria to express biofilm is dependent on certain factors amongst which is the availability of a surface material or substratum for successful bacteria attachment, (Giao & Keevil, 2014).

## **RESEARCH METHOD**

### **Study Area**

The study area for the research work is the bathrooms of Rivers State, Nigeria. The bathrooms in Nigeria are associated with dampness conditions due to the state's geographical location (coastal lines) (Izeogu & Salau, 1985). The study area Port Harcourt, Rivers State is located over 64 kilometers from the Atlantic Ocean. It is thus close to deep water and may be responsible for the proliferation of *Serratia marcescens* in the bathroom. Bathroom temperature in Rivers State, Nigeria is ranged from 26 to 37 degrees centigrades' which more or less determines the presence of *Serratia marcescens* (Romanowski et al., 2011).

### **Towel Swap Sample Collection and Preparation**

Forty (40) differently, used towels were collected from volunteered households. The towels were collected from twenty (20) different homes (bathrooms) and the swab was obtained. In obtaining the swab, a swab stick was moistened on a sterile normal saline and swabbed on the surface of the collected towels severally and after which inoculated into a freshly, prepared sterile peptone water (broth culture) in a test tube. The test tube was incubated for 18 hours for the growth of viable cells (Enciso-Moreno et al., 2004).

### **Media Preparation**

Media preparation involved weighing the required amount of agar needed and dissolving them in water, autoclaving as instructed by the manufacturer before use. Twelve grams (12g) and seven (7g) of MacConkey and Nutrient agar respectively, were dissolved in 250 ml distilled water for isolation and confirmation of *Serratia marcescens*. For biofilm formation of the recovered cell, Congo red agar was prepared using: Brain heart infusion agar (8g), Sucrose (12g), and Congo red indicator (2g), all dissolved in a 250ml distilled water (Amadi-Ikpa et al 2022; Freeman et al., 1989).

### **Bacteriological Analysis for *Serratia marcescens***

Analysis entailed inoculating the swabbed component into freshly prepared MacConkey agar medium, with a sterile pipette by the transfer of an inoculum (0.1ml) of the broth culture into a freshly prepared MacConkey media. The media was incubated at an ambient temperature for 24 hours. Isolation of pure cultures of *Serratia marcescens* after 24 hours was done based on the appearance of red colonies on the media background (plate) (Franco-Duarte et al., 2019). A confirmatory test for *Serratia marcescens* was carried out by streaking the colony onto a freshly prepared Nutrient agar medium. An inference of red-pigmented colonies after 24 hours at room temperature indicated *Serratia marcescens* while the reverse indicated no *Serratia marcescens* (Franco-Duarte et al., 2019).

### **Morphological / Colonial Characterization of *Serratia marcescens***

Colonial characterization involved a macroscopic description of the colony appearance of *Serratia marcescens* concerning color, size, elevation, edge, opacity, and shape. Morphological characterization involved a Gram staining procedure as determined by Franco-Duarte et al. (2019). Morphological characterization as carried out involved heat fix of the colony on a clean macroscopic slide via flame side to side. Thereafter, the slide was stained with Crystal-violet, iodine, ethanol and safranin at different time intervals with each stages washed off with water simultaneously. This was then viewed under a light microscope. A pink colored appearance indicated *Serratia marcescens*(Franco-Duarte et al., 2019).

### **Biochemical Identification of *Serratia marcescens***

The following biochemical test, Voges-Proskauer, Citrate, Indole, Catalase, Capsule, and motility assay were carried out (Franco-Duarte et al., 2019; Enciso-Moreno et al., 2004).

#### 1. Voges Proskauer Test

A loopful of the bacterium was inoculated into a 10ml sterile Voges Proskauer broth medium prepared according to manufacturer's instructions. The tube was then incubated at 35 - 37 degrees centigrade for 48 hours after which 0.6ml of 5 percent - naphthol and 0.2ml of 40 percent Potassium Hydroxide reagent was added to the broth culture. An inference of red coloration indicated Voges Proskauer positive test while an absence of red coloration did not indicate Voges Proskauer test.

#### 2. Citrate Test

Citrate analysis tested the ability of the isolate to utilize sodium citrate as its sole source of carbon and inorganic ammonium salt. Simmon citrate agar was prepared in a capped tube according to the manufacturer's instructions. A sterile wire loop was used to pick a loop full of the bacterium onto the agar slant surface and the tube incubated at 37 degrees centigrade for 24 hours. An inference of a changed color from green to blue indicated citrate utilization whereas: no change in color did not indicate citrate usage.

#### 3. Indole Test

This test was used to determine the ability of the isolate to split the amino acid tryptophan to form pyruvic acid, ammonia and indole using the enzyme tryptophanase. A loopful of the bacterium was inoculated into sterile peptone water and incubated at 37 degrees centigrade for 48hrs. Thereafter, 0.3 - 0.5 ml of Kovac's reagent was added using a Pasteur's pipette. An inference of a red ring on the medium indicated an indole utilization while a yellow ring indicated the absence of indole.

#### 4. Catalase Test

To determine the capacity of the isolate to express catalase, that is the ability to breakdown Hydrogen Peroxide into Oxygen and Water. A loop-full of the bacterium was inoculated into a clean slide containing Hydrogen Peroxide and observed. Inference was observed with a visible effervescent, an indication of catalase utilization while an absence indicated poor or no effervescent.

#### 5. Motility Test

Semi-solid nutrient agar was used for this test. The media were prepared, and introduced into a test tube and the test organisms picked with a sterile straight wire into the media by stabbing.

Thereafter, the medium was incubated at 37 degrees centigrade for 24 - 48 hours. An inference of growth in a diffused form, from the line of stab through the medium indicated a positive result, whereas growth only along the line of stab indicated a negative result.

#### 6. Capsule

Capsule staining test was done to determine the presence of a capsule in the bacterium. The procedure was carried by adding a few drops of crystal violet onto the test bacteria on a clean microscopic slide, then stirred and viewed under a light microscope. An inference of a light blue appearance on the microscope signified encapsulated cell, while the reverse signified the cell was not capsulated.

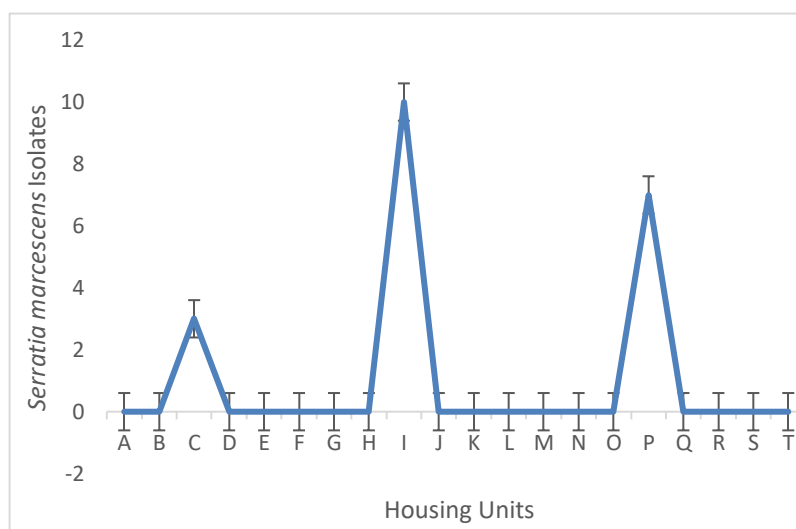
#### Biofilm Formation

This test was done to figure out the formation of *Serratia marcescens* biofilm. The *Serratia marcescens* isolates were subjected to Congo red agar medium, the agar medium commonly used to determine biofilm formation properties of bacteria (Freeman et al., 1989). The Congo red agar as employed determined qualitatively, *Serratia marcescens* as described by Amadi-Ikpa et al. (2022) in their study. The test by Amadi-Ikpa et al. (2022) adopted the streak method where *Serratia marcescens* isolates were introduced into a Congo red media that is composed of Brain Heart Infusion agar, supplemented with agar-agar, sucrose and Congo red stain. The inoculum was streaked in a zig-zag pattern continuously with a sterile wire loop. The media after inoculation was incubated at a temperature of 37 degrees centigrade for 18 – 24 hours. Black colonies with a dry crystalline background developed; indicating biofilm formation while an absence of black colonies indicated no biofilm formed (Amadi-Ikpa et al., 2022), (Budeli et al., 2018).

### FINDINGS AND DISCUSSION

#### Spatial Distribution/Variation of *Serratia Marcescens* on Body Towels

The histogram, below showed the trend of *Serratia marcescens* isolates obtained from forty (40) body towels in twenty (20) homes. The presence of *Serratia marcescens* isolates recovered from the body towels, was observed from three housing units out of the twenty homes. The three homes or points showed a bacteria count of  $3 \times 10^1$ ,  $1 \times 10^2$  and  $7 \times 10^1$  CFU (Coliform Forming Unit) per swab for house unit/points C, I, and P respectively. The house units/points A, B, E, F, G, H, J, K, L, M, N, O, Q, R, S, and T did not show *Serratia marcescens* growth/development on the body towels.



**Figure1: Spatial Distribution/Variation of *Serratia marcescens***

**Colonial/Morphological Characteristics of *Serratia marcescens* Isolates**

Colonial classification of *Serratia marcescens* isolates as seen in the chart below, showed the appearance of *Serratia marcescens* in terms of color, size, elevation, edge, opacity, shape, and Gram's reaction.

**Table: 1 Colonial/Morphological Characteristics of *Serratia marcescens* Isolates**

Bacteria	Color	Size	Elevation	Edge	Opacity	Shape	Gram React.
<i>Serratia marcescens</i>	Red	Small	Low	Curve	Opaque	Round	-

Note- = Negative

**Biochemical Characterization of *Serratia marcescens***

The biochemical screening of the *Serratia marcescens* was able to show key confirmatory results. The chart below reported/revealed the biochemical features of *Serratia marcescens*.

**Table: 2 Biochemical Characterization of *Serratia marcescens***

Motility	Catalase	Indole	Citrate	Voges Proskauer	Capsule	Bacteria
+	+	-	+	+	-	<i>Serratia marcescens</i>

Note: Positive = +, Negative = -

**3.4 Biofilm Formation by *Serratia marcescens***

The Figure 2, showed the swab results for the investigation for the biofilm capacity of *Serratia marcescens*. The result showed that most cells or colonies were actively growing in a biofilm. Thus, *Serratia marcescens* biofilm-forming capacities were widespread and actively growing.



**Figure2: Biofilm Formation by *Serratia marcescens***

The counts of *Serratia marcescens* at a peak of  $1 \times 10^2$  CFU per swab in one of the homes under study is not fatal to the user of such towel, as studies by Wilkowske et al. (1970) showed fatal concentration of *Serratia marcescens* counts between  $10^6$  to  $10^8$  CFU/ml on blood bacteria screened for (*Serratia marcescens*) septicemia in Japan. The incident however resulted in death (Wilkowske et al., 1970). Similarly, in a study carried out by Abdul and Hassan (2012), the *Escherichia coli* and *Staphylococcus aureus* counts in towel samples from school hostels were  $4.00 \times 10^2$  and  $8.23 \times 10^2$  CFU (Colony forming Unit) per ml (mill) respectively, which accounts to a higher bacteria load about *Serratia marcescens* obtained. This might likely be attributed to the swab method of analysis and the status of *Staphylococcus aureus* as a normal flora of the skin surface. The towel's frequent contact with the skin may have initiated the large concentration of *Staphylococcus aureus*. The less and high counts of *Escherichia coli* as compared to *Staphylococcus aureus* and *Serratia marcescens* were not questionable due to the closeness or presence of towels to the toilet/bathroom, where faces are passed and poor practice on the abusive use of towels (Wilkowske et al., 1970). The discovery of *Serratia marcescens* on body towels hung in the bathroom, could be likened or attributed to the contamination of toilet seats by *Shigella sonnei* following flushing of feces contained  $10^7$  CFU/gram of *Shigella sonnei* (Schreck et al., 2021). The true occurrence of *Serratia marcescens* in any environmental sample could be estimated (Christina et al., 2019), where the colonial/ morphological character of *Serratia marcescens* as stated in this study quite agreed with studies by Bin et al. (2011). Bin et al. (2011) observed the colonies of *Serratia marcescens* were colored red and round on nutrient agar at 48 hours of incubation and 28 degrees centigrade temperature. Similarly, classical bacteriological tests showed that they were Gram-negative, motile, and rod-shaped bacterium. The presence of *Serratia marcescens* in body towels placed in the bathroom is, due to their presence in washbasins, water closets etcetera in the bathroom (Sarvikivi et al., 2004). Sarvikivi et al. (2004) reported the clustering of *Serratia marcescens* in the washbasin of a neonatal intensive care unit of some hospitals. Similarly, studies by Twumwaa et al. (2020) have shown that the damp environment of an average bathroom allows germs on towels to thrive whereas other items in the house such as toothbrushes, and towels have been implicated to harbor diverse bacteria. Abney et al. (2021) were able to show that *Escherichia coli*, a Gram-negative bacteria was isolated from flush handles, toilet seats, and the underside of the toilet lid. In a different circumstance, according to Cristina et al. (2019), *Serratia marcescens* is mostly, detected in neonatal intensive care units of hospitals. Also, their presence in the bathroom in this present study may be attributed to the use of soap and shampoo as reported by Madani et al. (2011). Although, cleaning the bathroom with detergent or soap may spread the bacterium throughout the bathroom, as soap is a medium that encourages its proliferation. Hence, Khanna et al. (2013), pointed out that *Serratia marcescens* could be isolated from soap dispensers. However, the removal of soap dispensers could help manage the spread of the bacteria. According to the report by Madani et al. (2011), *Serratia marcescens* contaminate and degrade baby shampoo thereby, caused an outbreak among newborns after use. The shampoos must have been contaminated due to their placement/stay in the bathroom. *Serratia marcescens* isolates formed biofilms, principally, the ability of *Serratia marcescens* to form biofilm is due to its motile property where the structural presence of flagella enabled it to move to receptor sites or a substratum (Suzina et al., 2011). These further show the spreading factor and airborne property the bacterium possess in the contaminating towel. This result agreed with a study carried out by Stewart et al. (2012), where biofilms were observed on platelet as a result of *Serratia marcescens* presence. The result that biofilm is formed; probably on the surface of the body towel, strongly suggests that the towels provide attachment or receptor sites for the bacteria biofilm formation and growth (McClean et al., 2012). The build-up of *Serratia marcescens* biofilm on towels may have also resulted from the biofilm persistence property. Exposure of these towels to *Serratia marcescens* is possible due to poor cleaning habits in specific areas within the bathroom as well as negligence on the part of users on the hygienic state of their body towels. Thus the harbor of these bacteria in the towel may have resulted during the flushing of feces where the bowl harbors the bacteria. *Serratia marcescens* may have ejected from these areas of the bathroom (bowl/urinal) and got transmitted through aerosols to surfaces (body towels) (Schreck et al., 2021). Thus, a significant effect specifically on body towels, presents a hostile community for the possible degradation of keratin as the towel makes contact

with skin allowing possible transmission of the bacteria. Thus, the environmental monitoring of contact surfaces or premises ensured the cleanliness of surfaces which are noted vehicles for microbial transition (Ramandi & Asgharian, 2020). Hence, the significance of this study lies in the keratolytic property expressed by *Serratia marcescens* (Fuke et al., 2018) and by implication, the degradation of human skin when body towels hung in bathrooms are used.

## CONCLUSION

The prevalence of *Serratia marcescens* in bathrooms presents a clear threat to towel users who hang their towels in the bathroom. Thus, the study concluded and noted that the presence of *Serratia marcescens* capable of forming a biofilm on body towels hung in the bathroom could have health implications for users due to its keratolytic property. This challenge on body towels hung in the bathroom calls for concern and urgent attention. Regular cleaning of the surface wall of tiles and gut, basin etcetera, and other sanitary wares are advised aimed at controlling the colonization by *Serratia marcescens*. There is need for constant surveillance to support effective measures of hygiene aimed at preventing the spread of the bacterium, even at its opportunistic property of been pathogenic on immune-compromised users.

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