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Phenotypic determination of virulence attributes of *S. aureus* and *P. aeruginosa* isolated from clinical specimens in Port Harcourt, Rivers State

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Abstract
Staphylococcus aureus and *Pseudomonas aeruginosa* are common opportunistic pathogens frequently encountered in clinical settings, responsible for a wide range of infections. This study aimed to determine the pathogenicity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from clinical specimens in Port Harcourt, Rivers State, Nigeria. A total of 589 specimens: wounds (133), ear swab (107), urine (170) and stools (184) obtained from patients in two University Teaching Hospitals were cultured on Mannitol salt and Cetrimide agar plates. The bacteria were identified by their morphological characters and biochemical tests. Phenotypic characterization of virulence factors was determined, including motility and pyocyanin production. Results showed that a total number of 101 *Staphylococcus aureus* and 56 *Pseudomonas aeruginosa* isolates were identified from the specimens. Data indicated that both *S. aureus* and *P. aeruginosa* exhibited an array of virulence determinants, contributing to their ability to colonize and cause infections. About 85.7% and 75% of *S. aureus* from wound and stools were biofilm formers, 66.7% and 85.7% of *S. aureus* from wound specimens were coagulase-positive, 85.7% and 100% of *S. aureus* were beta-haemolytic while 33.33% and 85.71% were lecithinase positive. The range of biofilm production, beta-haemolysis, lecithinase, swarming and pyocyanin production by *P. aeruginosa* was 75-84%, 66.7-75%, 25-50%, 100% and 100%, respectively. The findings of this study revealed that *S. aureus* and *P. aeruginosa* possess varying degree of virulence and could be pathogenic. This highlights the need for effective infection control strategies to reduce the prevalence of these suspected pathogens in the community, and underscores the importance of proper prescription of antibiotics.

Keywords: Virulence, *P. aeruginosa*, *S. aureus* clinical specimen

Introduction
The relationship between pathogen and host is reciprocally devastating during acute infection, as bacteria produce a variety of cytotoxic molecules that impair host cellular processes, while bacteria encounter immune system responses such as the production of antimicrobial compounds and reactive oxygen, as well as enhanced phagocytosis (Moradali *et al*., 2017) [49]. The ability of an organism to infect the host and spread disease is referred to as virulence. These substances known as virulence factors either have a secretory, membrane-related, or cytosolic character that help the bacterium colonise the host at the cellular level (Sharma *et al*., 2017) [26] thus, enhancing their pathogenicity.
Pseudomonas aeruginosa an aerobic and oxidase positive gram-negative bacterium is regarded as one of the most significant causes of hospital acquired infections, particularly in intensive care units. Despite not often being pathogenic, this bacterium can cause opportunistic infections in people with weakened immune systems, such as ICU patients (Zarei *et al*., 2018) [30]. Swimming and swarming with flagella, as well as twitching with type 4 pili, are all related with virulent characteristics in *P. aeruginosa* (Winstanley *et al*., 2016) [34]. Flagellar and/or other motility components mediate recognition and stimulation of signaling pathways that elicit inflammatory reactions and phagocytosis by murine or human macrophages, making a motile cell easily identifiable by the host immune system (Winstanley *et al*., 2016) [34]. Many harmful bacteria, such as *P. aeruginosa*, evade stressors and harsh circumstances by switching to a sessile lifestyle along with diminished virulence. They lose their mobility and cling to surfaces, forming cellular aggregations or microcolonies that are protected from the environment by extracellular polymeric substances (EPS) (Moradali *et al*., 2017) [49].

More so, *P. aeruginosa* is renowned for developing robust biofilms that are highly resistant to antibiotics, disinfectants, and host defenses (Lee and Yoon, 2017; Yan and Wu, 2019) [35, 36], impairing bacterial clearance and leading to the establishment of highly recalcitrant chronic infections that are major medical problems.

Staphylococcus aureus is another notorious and pervasive bacterial pathogen that is responsible for hundreds of thousands to millions of more serious, invasive infections each year and an uncountable number of simple skin infections (Klevens *et al*., 2007; Rasigade *et al*., 2014) [1, 4]. According to Tong *et al*. (2015) [5], *S. aureus* is a major cause of pneumonia and other respiratory tract infections, cardiovascular infections, surgical site infections, infections of prosthetic joints, and nosocomial bacteremia. Similar to *P. aeruginosa*, *S. aureus* produce biofilm and ability to generate biofilm is one of its defense mechanisms (Reda *et al*. 2017) [37]. *S. aureus* secretes soluble virulence compounds that can disrupt epithelial barriers, facilitate immune evasion, and increase inflammation in addition to its ability to form biofilms (Moran *et al*., 2019) [38], raising the value of effective treatments. *Staphylococcus* spp. produce cytolytic exotoxins, which cause the lysis of the host's targeted cell by forming tiny pores in the plasma membrane (Shrestha *et al*., 2018) [39]. The paucity of data on the virulent attributes of *S. aureus* and *P. aeruginosa* from clinical specimens in Rivers State justifies the present study. Due to the rate of infection caused by these opportunistic pathogens, elucidating their virulence would aid in treatment or control in the hospitals and environment.

Materials and Methods

Specimen Collection

The study was a cross-sectional study and specimens were collected randomly within the period of May 2022 to April, 2023. A total of five hundred and eighty-nine specimens comprising of wounds (133), ear swab (107), urine (170) and stools (184) were collected from the microbiology laboratory of the Rivers State University Teaching Hospital and the University of Port Harcourt Teaching Hospitals. All specimens were collected according to standard methods (Cheesbrough, 2006) [40]. Wound swabs were collected as described by Kassam *et al*., (2017) [41]. Before obtaining the specimen, the wound was thoroughly cleaned with 60–120 mL of sterile normal saline to prevent contamination of the swab with skin bacteria, pus, or necrotic tissue. The wound's surface was cleaned of excess saline using sterile gauze, and pus swabs were obtained by swabbing the wound's centre with a sterile swab. Deep incisions were made with a cotton swab, saturated with sterile normal saline, and immediately placed into a tube containing transport media for preservation. Similarly, the ear swabs were collected as described by Motayo *et al.* (2012) [42] from patients by inserting swab sticks moistened with normal saline gently into the ear of patients without touching the outer skin. These tubes were sent to the microbiology laboratory, Rivers State University for immediate analysis

Isolation of *S. aureus* and *P. aeruginosa*

Swab specimens were cultured as described by previous studies (Motayo *et al.*, 2012; Kassam *et al*., 2017) [41]. On reaching the Microbiology Department, swab sticks were swabbed onto the surface of aqueously prepared sterile mannitol salt and cetrimide agar plates while stool and urine specimens were inoculated onto mannitol and blood agar by streaking on the surface of the agar plates for the isolation of *Staphylococcus* and *Pseudomonas* isolates, respectively (Cheesbrough, 2006) [40]. The inoculation was done in duplicates and plates were incubated at 37 ℃ for 24-48 hours. After incubation, plates were read and those showing golden yellow colonies on mannitol salt agar were isolated by streaking on freshly prepared mannitol salt agar and nutrient agar plates while those showing blue-green colonies on cetrimide agar were subcultured onto nutrient agar plates. Incubation at 37 ℃ for 24 hours followed.

Preservation and Identification of Isolates

The pure staphylococcal and *Pseudomonas* isolates were preserved in bijou bottles containing 3mL sterile glycerol (10% v/v) and were stored frozen in the refrigerator while another set was preserved in agar slant and was refrigerated. These pure isolates were used both for the identification process and other tests. The isolates were identified based on their colonial morphology and biochemical tests (Ihechu *et al*., 2023; Robinson *et al*., 2023) [21, 24].

Phenotypic Determination of Virulence

The virulence determined included the presence of enzymes (coagulase, lecithinase and haemolysin), biofilm production, pyocyanin production and presence of flagella (motility). The tests are described below;

Coagulase Test

This test was used to determine whether coagulase was present (an enzyme that coagulates blood). It helps to distinguish between *Staphylococcus epidermidis* and pathogenic *Staphylococcus aureus*. On a clean grease-free microscope slides, a colony of the test isolate was emulsified on a drop of saline on one end while the other end has only saline. After which, a drop of human plasma was applied on both ends. The both ends were observed immediately for clumping. Agglutination (clumping) of the blood plasma within 10-15 seconds was read as a positive test (Prescott *et al*., 2011) [12].

Lecithinase Test

The purpose of this experiment was to determine whether the isolates could create the enzyme lecithinase, also known as α-toxin, which combines with lecithin in egg-yolk medium to form an iridescent layer that denotes lypolysis and an opalescence that shows lecithinase activity. The isolates were applied to the egg yolk medium in a single line by streaking with a sterile wire loop. The plates were incubated at 37 ℃ for 24 hours and examined for opalescent halo surrounding around the inoculum. Appearance of a white, opaque, diffuse zone that extends into the medium surrounding the colonies indicated a positive test while the absence of a white, opaque zone extending from the edge of the colony signified a negative test (Oladipo *et al*., 2008) [32].

Haemolysis Test

Investigations were made into the isolates' hemolytic activity. This was done to determine whether the bacterial isolates could break down red blood cells. The test isolates were streaked on to a blood agar (Sagars, 2015) [42]. The plates were incubated at 37 ℃ for 24-48 hours after which the plates were read for the presence of beta (complete

haemolysis), gamma (no haemolysis) or alpha (partial haemolysis) haemolysis (Prescott *et al*., 2011) [12].

Biofilm Test

Congo red agar (CRA) was the solid media used in the biofilm plate test. With the use of this technique, it is possible to directly analyse the colonies and distinguish between slime-forming strains (which show up as black colonies on red agar) and non-slime-forming strains (red-coloured colonies). The bacterial isolates were grown on prepared sterile CRA plates that were made by mixing 1 L of Brain Hart Infusion agar (BHI) with 0.8 g of Congo red and 36 g of glucose and the plates were incubated for 24 hours at 37 °C. Slime-producing strains were distinguished from non-slime-producing isolates (red smooth colonies) by the presence of rough black colonies (De Castro Melo *et al*., 2013) [43].

Synthesis of pyocyanin pigment

This was done according to El-Fouly *et al.* (2015) [10]. In 250 ml conical flasks with 50 ml of glycerol-supplemented nutrient broth medium (GSNB), various *P. aeruginosa* strains were grown and incubated at 37 °C for four days for determination of pyocyanin production.

Motility test

This test which determines whether bacteria possessed flagella or not was done as described Charkraborty and Nishith, (2008) [44]. It was performed by stabbing the test isolate into half strength nutrient agar medium and incubated at 37 °C for 24 hours. Diffuse, hazy growths that spread throughout the medium rendering it slightly opaque represents a positive / motile organism while growth that is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent represents a negative test/ non-motile organism.

Results

Results of the virulence traits of *S. aureus* as demonstrated in biofilm, coagulase, haemolysis and lecithinase is presented in Tables 1 to 3. Results showed that 100, 25.0, 61.5 and 50% of the isolates from wound, stool, ear and urine specimens produced biofilms. For haemolytic activity, all wound specimens were haemolytic while 75, 76.9 and 70% from stool, ear and urine specimens were haemolytic. The prevalence of *S. aureus* with lecithinase activity showed that 33.3, 37.5, 61.5 and 80% were lecithinase positive while 66.7, 37.5, 46.2 and 70% were coagulase positive (Table 1).

The data in Table 2 showed that 85.7, 25.0, 36.4 and 34.3% of the isolates were biofilm producers while 85.7, 66.7, 81.8 and 74.3% were beta-haemolytic. The prevalence of lecithinase and coagulase activity showed that 85.7, 83.3, 81.8 and 57.14 were positive for lecithinase while 85.7, 41.7, 54.5 and 68.6 were coagulase positive.

In Table 3, Results further showed that 100% and 85.7% from wounds in RSUTH and UPTH, respectively were biofilm formers. This was followed by isolates from the stool specimens which showed that 75% of *S. aureus* from both hospitals were biofilm formers while the third specimen with high biofilm activity were isolates from the ear specimens. Although about 50% of *S. aureus* isolates from urine specimens collected from RSUTH were biofilm formers, only a lesser percentage of 34.3% of the isolates from similar specimens collected from patients in UPTH were positive. In UPTH, the prevalent coagulase positive *S. aureus* was from wound specimen while the highest in RSUTH was recorded from urine specimen. Results further showed that most of the isolates from all the specimens from both hospitals were coagulase positive as well as lecithinase positive. The urine specimens from RSUTH had higher prevalent lecithinase activity than other specimens from same location but lower to those isolated from wound specimens of UPTH.

Table 1: Prevalence of Virulent producing *S. aureus* in Clinical Specimens from RSUTH

Virulence	Specimens			
	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]
Biofilm	3 (100)	2 (25.0)	8 (61.5)	6 (50)
Haemolysis	3 (100)	6 (75.0)	10 (76.9)	7 (70)
Lecithinase	1 (33.3)	3 (37.5)	8 (61.5)	8 (80.0)
Coagulase	2 (66.7)	3 (37.5)	6 (46.2)	7 (70)

Table 2: Prevalence of Biofilm producing *S. aureus* in Clinical Specimens from UPTH

Virulence	Specimens			
	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]
Biofilm	6 (85.7)	3 (25.0)	8 (36.4)	12 (34.3)
Haemolysis	6 (85.7)	8 (66.7)	9 (81.8)	26 (74.3)
Lecithinase	6 (85.7)	10 (83.3)	9 (81.8)	20 (57.14)
Coagulase	6 (85.7)	5 (41.7)	6 (54.5)	24 (68.6)

Table 3: Comparison of the Prevalence of *S. aureus* in Clinical Specimens in Both Hospitals

Virulence	RSUTH				UPTH			
	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]
Biofilm	3 (100)	2 (25.0)	8 (61.5)	6 (50)	6 (85.7)	3 (25.0)	8 (36.4)	12 (34.3)
Haemolysis	3 (100)	6 (75.0)	10 (76.9)	7 (70)	6 (85.7)	8 (66.7)	9 (81.8)	26 (74.3)
Lecithinase	1 (33.3)	3 (37.5)	8 (61.5)	8 (80.0)	6 (85.7)	10 (83.3)	9 (81.8)	20 (57.14)
Coagulase	2 (66.7)	3 (37.5)	6 (46.2)	7 (70)	6 (85.7)	5 (41.7)	6 (54.5)	24 (68.6)

Results of the virulence traits of *P. aeruginosa* as demonstrated in biofilm, haemolysis and lecithinase is presented in Tables 4 to 6. Results of the prevalence of *P. aeruginosa* with biofilm production in the different specimens showed that 84% and 75% of *P. aeruginosa* isolates from wounds and urine were positive biofilm formers. The isolates from the ear specimens showed that 50% and 62.5% of *P. aeruginosa* from both hospitals were biofilm formers while 72.7% of the isolates from wound specimens in UPTH were biofilm positive isolates (Table 6). The prevalence of *P. aeruginosa exhibiting* haemolytic activity showed that isolates from the ear, stool, and wound specimens from RSUTH were 25%, 66.7% and 61.5%, respectively (Table 4) while the prevalence of the isolates of

P. aeruginosa exhibiting haemolytic activity from the ear, urine and wound specimens collected from UPTH was 62.5%, 75% and 36.4%, respectively (Table 5).

The prevalence of lecithinase activity by *P. aeruginosa* isolates showed that only 50% and 30.8% of the isolates from ear and wound specimen possessed the enzyme lecithinase especially for specimens collected from RSUTH while only 25%, 25% and 18.2% from ear, urine and wound specimens possessed the lecithinase enzyme in specimens collected from UPTH (Table 6).

Results of the swarming motility and production of pyocyanin by *P. aeruginosa* showed that all (100%) of the isolates were motile and possessed the pyocyanin pigment.

Table 4: Prevalence of Biofilm producing *P. aeruginosa* in Clinical Specimens from RSUTH

Virulence	Specimens			
	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]
Biofilm	11 (84.6)	1 (25.0)	2 (50.0)	0
Haemolysis	8 (61.5)	2 (66.7)	1 (25.0)	0
Lecithinase	4 (30.8)	0	2 (50)	0
Swarm motility	13 (100)	0	4 (100)	0
Pyocyanin	13 (100)	0	4 (100)	0

Table 5: Prevalence of Biofilm producing *P. aeruginosa* in Clinical Specimens from UPTH

Virulence	Specimens			
	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]
Biofilm	16 (72.7)	0	5 (62.5)	3 (75.0)
Haemolysis	8 (36.4)	0	5 (62.5)	3 (75.0)
Lecithinase	4 (18.2)	0	0	1 (25.0)
Swarm motility	22 (100)	1 (100)	8 (100)	4 (100)
Pyocyanin	22 (100)	1 (100)	8 (100)	4 (100)

Table 6: Comparison of the Prevalence of *S. aureus* in Clinical Specimens in Both Hospitals

Virulence	RSUTH				UPTH			
	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]	Wound [n (%)]	Stool [n (%)]	Ear swab [n (%)]	Urine [n (%)]
Biofilm	11 (84.6)	1 (25.0)	2 (50.0)	0	16 (72.7)	0	5 (62.5)	3 (75.0)
Haemolysis	8 (61.5)	2 (66.7)	1 (25.0)	0	8 (36.4)	0	5 (62.5)	3 (75.0)
Lecithinase	4 (30.8)	0	2 (50)	0	4 (18.2)	0	0	1 (25.0)
Swarm motility	13 (100)	0	4 (100)	0	22 (100)	1 (100)	8 (100)	4 (100)
Pyocyanin	13 (100)	0	4 (100)	0	22 (100)	1 (100)	8 (100)	4 (100)

Discussion

The ability of an organism to cause infection is to a larger extent dependent on its virulent factors. These refer to the factors or molecules that aid the bacterium or microorganisms to colonize the host at cellular level (Sharma *et al*., 2017) [26]. According to Vandenesch *et al.* (2012) [27], a bacterium potential to cause disease is linked to a vast range of virulence factors that allow colonisation and persistence, spread within the host, and immune system evasion. The set of virulence factors needed to generate disease is expected to be greatly dependent on the site of infection [for example, skin and soft tissue infections (SSTI) versus infective endocarditis] (Vandenesch *et al*., 2012) [27]. In the present study, the *S. aureus* and *P. aeruginosa* were all screened for the formation of biofilm, lecithinase, coagulase, haemolysin, pyocyanin and swarm motility. Based on the findings, most of the isolates (*S. aureus* and *P. aeruginosa*) from the different specimens were biofilm formers. Formation of biofilm by microorganisms have been reported to contribute to bacterial virulence as well as aid in antimicrobial resistance. Biofilms are immobile microbial communities that colonise and grow on the surfaces of medical implants like sutures, catheters, and dental implants. They produce extracellular polymeric substances that can only be removed in order to treat the infections they cause, which makes treatment expensive and increases the risk of patients developing mental illness (Hoiby *et al*., 2011) [45]. More so, due to their ability to withstand antibiotics, host defense mechanisms, and other external stresses, bacterial biofilms are a serious global health concern that contributes to persistent chronic infections (Divakar *et al*., 2019) [15]. Thus, the production of biofilm by the *S. aureus* and *P. aeruginosa* isolates in the present study implied that they could possess antimicrobial resistance and this is a great public health challenge as they could cause prolonged hospital stay. The high biofilm production exhibited by *S. aureus* in the present study agreed with Gaire *et al.* (2021) [17] who reported that all *S. aureus* isolated from clinical specimens were biofilm formers. More so, there was disparity in the response of the isolates to biofilm production especially the variation witnessed in the specimens as well as the locations. This is consistent with Liu *et al*., (2020) [9] who reported that biofilm formation could be influenced by many factors including nutrient availability, environment, geographical origin, specimen type, genetic make-up of the organism and adhesion characteristics. Production of biofilm by *Pseudomonas aeruginosa* isolates have been reported by previous studies (Wiley *et al*., 2012; Huse *et al*., 2013) [7, 8]. Mittal *et al.* (2009) [23] opined that due to their propensity to produce persistent and recurrent infections, biofilms help *P. aeruginosa* become pathogenic and the propensity to cause infection could be linked to the production of biofilm.

The presence of coagulase enzyme in most of the *S. aureus*

isolates from the different specimen supports the claim that these isolates could be virulent. Coagulase secretion is one of the key virulence strategies used by *Staphylococcus* sp to get past the host defence system. Coagulases are secretory proteins that activate prothrombin to cause blood clotting (Emilia *et al*., 2018 [46]. Thus, clothing of the blood helps them not to be detected by the host defence system (phagocytes). Cheng *et al*. (2010) [13] in their study also reported that coagulase aid *S. aureus* in causing diseases as well as protecting them from host phagocytes. Thus, formation of clots by these isolates could mean that they could evade host tissues and cause diseases.

In the present study, a very high percentage of the *S. aureus* and *P. aeruginosa* isolates produced beta-haemolysis. They completely produced clear zones on the red blood cells and their ability to haemolyze blood could imply that they are virulent. It has been hypothesised that haemolysin increases virulence by increasing the iron available, mediating the toxic effect on leucocytes and other nucleated cells, potentiating the effect of endotoxin, and mediating serum resistance (Egbe and Enabulele, 2014) [16]. Haemolysin is amongst the pore forming enzymes that plays key role in dissemination of infection (Georgescu *et al*., 2016) [18]. Previous studies have reported the haemolytic activities of *S. aureus* and *P. aeruginosa* as virulent factor of these isolates (Egbe and Enabulele, 2014; Georgescu *et al*., 2016; Sharma *et al*., 2017; Vandenesch *et al*., 2012) [16, 18, 26, 27] and this agreed with the present study which showed that the isolates produced this enzyme.

One of the virulent factors that aids in invasion of tissues of host is lecithinase. The enzyme is known to breakdown red blood cells and also catalyse the disruption of phospholipids in the membranes of red blood cells (Prescott *et al*., 2011) [12]. Lecithinase has a toxic effect because it typically interacts with cell membranes, either by rupturing them and causing cell lysis or by destroying the phospholipids (Sharaf *et al*., 2014) [25]. Lecithinase lyses red blood cells, myocytes, fibroblasts, platelets, and leukocytes, according to Shetty *et al*. (2009) [47]. In the present study not all of the isolates of *S. aureus* and *P. aeruginosa* produced lecithinase but irrespective of this, the presence of the lecithinase enzymes in most of these isolates implied that they could be virulent. Swarming motility and pyocyanin production by *P. aeruginosa* isolates were also determined. Swarming of *P. aeruginosa* isolates have been regarded as a virulent factor especially as it enables the bacterium to migrate to other areas where they could cause infections. Swarming is a type of surface-associated motility in which a population of bacteria moves quickly and cooperatively across viscous semisolid surfaces (Kearns, 2010; Partridge and Harshey, 2013) [11, 6]. The production of biosurfactants like rhamnolipids helps with this type of movement in the opportunistic human pathogen *Pseudomonas aeruginosa* by reducing the surface tension between cells and the environment. This type of movement requires the presence of a functional flagella to mediate actual movement (Hou *et al*., 2019) [20]. Motility is closely linked to *P. aeruginosa* pathogenesis. This bacterium's motility allows it to colonise various environments, adhere to surfaces, and form biofilms (Yeung *et al*., 2009) [29]. The present study agreed with their study as all isolates were very motile and exhibited swarming activity. Thus, the ability of these isolates to swarm implied they possess flagellum and this could help in their virulence. Pyocyanin (PCN) is a blue green phenazine pigment produced in large quantities by active cultures of *Pseudomonas aeruginosa* (DeBritto *et al*., 2020) [14]. According to Zhou *et al.* (2022) [31] *P. aeruginosa* produces and secretes pyocyanin, a redox-active green pigment that contributes significantly to bacterial infections (Zhou *et al*., 2022) [31]. It is thought that PCN's low molecular weight and zwitterionic characteristics enable the toxin to quickly pass-through cell membranes. Although it has been claimed that PCN can cross biological membranes, its presence in systemic circulation has not yet been confirmed (Hall *et al*., 2016) [9]. Thus, the presence of PCN on all *P. aeruginosa* isolates denotes that they could be virulent thereby enhancing infectivity of the isolates on humans. A variety of roles in microbial ecology, including an important connection to the severity of *P. aeruginosa* infections, have recently been assigned to pyocyanin, which was previously disregarded as a secondary metabolite produced by bacteria (Hunter *et al*., 2012) [48]. Pyocyanin is easily able to pass through the host cell membrane due to its diffusible nature, where it can then engage in redox reactions with other molecules (Schwarzer *et al*., 2008) [2]. To give one example, it accepts electrons from NADH and then donates them to molecule oxygen to create reactive oxygen species (ROS), like hydrogen peroxide (H_2O_2) (Das *et al*., 2016) [3]. In addition to being a powerful inhibitor of other bacterial and fungal species present in the microbiome of the CF lung, the H_2O_2 produced by redox reactions has been shown to significantly alter the functions of host cells (Schwarzer *et al*., 2008) [2]. In CF patients, pyocyanin-mediated ROS oxidise host intracellular and extracellular reduced glutathione (GSH) to form glutathione disulfide or oxidised glutathione (GSSG) (Schwarzer *et al*., 2008) [2].

Conclusion

In conclusion, this study highlighted the diverse and multifaceted pathogenicity of *S. aureus* and *P. aeruginosa*, each driven by a unique set of virulence factors. The distinct clinical presentations of infections caused by these pathogens underscore the importance of early and accurate diagnosis for effective patient management. Understanding the pathogenic mechanisms of *S. aureus* and *P. aeruginosa* is crucial for developing targeted therapeutic strategies and preventive interventions to mitigate their impact on patient outcomes.

Conflict of Interest

Not available

Financial Support

Not available

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