Antimicrobial activity of *pseudomonas aeruginosa* against disinfectants Dettol, Savlon, Ethanol and Hydrogen peroxide

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***Abstract- Pseudomonas aeruginosa* is a gram- negative bacilli, rod shaped, motile. It causes infection in the adults as well as neonates, it is one of the most challenging bacteria as a nosocomial causing Pathogen. The immunocompromised patients are at a high risk. In the field of medical science, it is always challenging to eradicate the *P. aeruginosa* from the infected substratum. There are number of reasons which suggested that the disinfectants should be change periodically to avoid any kind of resistance toward. It was observed that the very commonly used disinfectants do not show high resistance towards them but uncommonly used disinfectants were effect toward the same group *P. aeruginosa.* There were five different disinfectants were selected against the *P. aeruginosa* to know the antimicrobial activity against the disinfectants. These disinfectants include Savlon (10% w/v), Lysol (10% w/v), Dettol (10% w/v), Ethanol (70% w/v) and Hydrogen Peroxide (3% w/v). The result show that the most commonly used disinfectants like Ethanol, Hydrogen peroxide and Dettol were not so much effective against all the P. aeruginosa strains isolated from different location. The maximum. Inhibition Zone was recorded in Savlon and Lysol at the same concentration, it also depends on the composition of these disinfectants. Out of 23 isolates only 5 strains show good result for the Dettol, ethanol and hydrogen peroxide.**

***Keywords*-disinfectants, Dettol, Savlon, Ethanol, Hydrogen Peroxide, pseudomonas aeruginosa.**

INTRODUCTION

Healthcare-associated infections (HAIs) are influenced by a multitude of factors, encompassing healthcare-associated elements such as the use of invasive devices, surgical procedures, and the inappropriate application of antimicrobial therapies [1,2]. Environmental aspects, including contaminated air-conditioning systems, also play a significant role, as do patient-related factors like the severity of underlying illnesses, the use of immunosuppressive agents, and prolonged hospital stays. Pseudomonas aeruginosa, a gram-negative, motile bacillus, is particularly notable in this context due to its capability for biofilm formation, which significantly enhances its pathogenicity [4,5,6]. This bacterium is an important opportunistic pathogen responsible for a variety of hospital-acquired infections, with its virulence and resistance posing substantial challenges in medical settings [7,8,9,10,11].

The pathogenicity and resistance of P. aeruginosa are primarily attributed to its ability to form biofilms, which protect the bacterial community from antibiotics, disinfectants, and the host immune response. This makes infections caused by P. aeruginosa difficult to treat, as very few antibiotics are effective against it. Additionally, contaminated disinfectant solutions can be a source of nosocomial infections, further complicating infection control efforts [12,13,14]. Various methods have been implemented to reduce the population of P. aeruginosa, including chemotherapy, immunization, sterilization, and disinfection. Among these, decontamination, disinfection, and sterilization have become fundamental components of infection control programs. Antiseptics and disinfectants are critical in these efforts, with antiseptics being biocides used on living tissues (such as healthcare personnel hand washes and surgical scrubs) and disinfectants being used on inanimate objects or surfaces [15,16,17].

The formulation of disinfectants, the level of organic charge, synergy, temperature, dilution rate, and examination methods all influence the antimicrobial activity of disinfectants. Biofilm formation by P. aeruginosa increases its resistance to antibiotics, disinfectants, and host immune responses, thereby complicating treatment and effective disinfection. This is not limited to human pathogens; several food-borne pathogens, including Listeria, Escherichia coli, and Salmonella, also have the ability to form biofilms. Research aimed at developing strategies to prevent and treat infections must consider the unique characteristics of biofilms. Additionally, effective disinfection protocols are necessary to eliminate biofilms in environments such as farms and food processing plants, as biofilms can serve as reservoirs for infectious agents [18,19].

The mechanisms of action of antiseptics and disinfectants on bacteria include uptake, lysis and leakage of intracellular constituents, perturbation of cell homeostasis, effects on model membranes, inhibition of enzymes, electron transport, and oxidative phosphorylation. The ability of P. aeruginosa to form biofilms on surgical instruments contributes to its high infection rates in operative patients, medical devices, and wounds. Common disinfectants used in household and hospital settings include Dettol, Savlon, Ethanol, Hydrogen peroxide, and Lysol. Ensuring proper use and understanding the factors affecting disinfectant efficacy are crucial for controlling the spread of infections caused by P. aeruginosa [20,21].

METHOD AND MATERIAL

Site of Sample collection

The samples were collected from the two hospitals ACMS Curesta and Medanta Hospital Irba. Ranchi Jharkhand. All the samples were collected from male, females, children's and also from the surfaces of the hospitals.

Sample collection:

All the samples were collected from the patients who were suffering from infection (urine, Pus, Blood etc.) and from the surfaces of the hospital which includes food tray and lift bottoms. Media were first autoclaved at 121℃ for 15 mints for 15psi to avoid any kind of contamination to the media. Once the media gets solidified the selected samples were swabbed or streaked on the MacConkey agar petri plate and incubated at 37℃ for 24 Hrs. after incubation the colonies were selected on the bases of color of the colony white non fermenting and pink fermenting bacteria. As the MacConkey agar is known as differential media.

Isolation of Pure Culture

The selected colonies were selected from the MacConkey agar media and then transferred to the Nutrient agar media. The inoculation was done aseptically. The Nutrient agar media was first prepared according to required quantity and then it was autoclaved at 121℃ for 15 minutes at 15psi. then it was poured in the petri plate and it was left for the solidification, once the solidification was done the cultures were inoculated and incubated for 24 Hrs. at 37℃. Then the cultures were morphologically examined and observed.

Morphology of the isolates:

After 24 hrs. of incubation the isolates were observed for the selection of the colonies the isolates were smooth and rough, irregular margin, flat elevation, opaque, some colonies were white, off white and some shows pigmentation. On the MacConkey agar colonies were grow white in color as it can't ferment the lactose. Due to which MacConkey agar is also known as differential media.

Gram Staining

The gram staining is one of the most important methods in the field of bacteriology which helps to identify the bacterial identification and observation throughout experiments. It also the first most important test to be performed. There were three different stains were used primary stain Crystal Violet and secondary stain Iodine followed with decolorizing agent and counter stain as Saffranine. After application of the gram staining isolates shows pink color which means its loses primary stain after application of the decoloring agent and takes only counter stain i,esaffranine due to which it appears pink in color. It is because it has outer membrane which is absent in gram positive bacteria. This is the reason gram staining is also known as differential staining.

Biochemical Tests

Biochemical test was performed to know the chemical nature of the specimens through which the results were made. The biochemical includes catalase test, TSI, Oxidase, nitrate reduction, starch hydrolysis etc. for the biochemical tests several different types of reagents were used which reacts with the metabolic activity of the bacteria and results were observed. All the test media were first measured according to the manufactures detailed then autoclaved at 121℃ for 15 mints at 15 psi. then it was allowed to cool, once the media were cooled down the selected inoculum were transferred from the freshly made cultures then labelling was done according to requirement. Once the inoculation was done in the respective test tubes or petri discs they were incubated to the incubator at 37℃ for 24 – 48 hrs. time depends on the criteria of the tests. Once the incubation period was completed the reagents were added to the test tubes and observed for the result. It is always important to take care about the contamination related precautions.

Urease test:

This test was performed to determine the production of urease enzyme by the microorganism, this test was performed in the test tube in which urease enzyme attack the carbon and nitrogen bond amide with the liberation of the ammonia. During the incubation period the isolates reduces ammonia which increase the pH of the media due to which phenol red changes from yellow color to the red color or pink color, which indicates the positive result and no changes color of the media shows the negative result.

Methyl Red test:

This test determines the ability of microorganism to fermenting with the production of acid as end products. The isolated microorganisms were inoculated in Methyl Red-VogesProskauer broth. All the inoculated were incubated at 37℃ for 24 hrs. After incubation, 5-6 drops of methyl red reagent were added to the broth/media. A Red color of medium indicates positive test, while no color change shows negative result[1].

VogesProskauer Test:

This test determines the capability of microorganism to produce non-acidic end products such as ethanol and acetoin from the organic acid. The isolated microorganisms were inoculated in Methyl Red VogesProskauer broth. All the inoculated were incubated at 37C for 24 hrs. After incubation, 12 drops of freshly prepared VP-reagent I (naphthol solution), 2-3 drops of VP reagent II (40% KOH) were added in all the inoculated and in controls. Development of crimson to pink (red) color indicated positive test no change in color indicated negative test.

Indole Test:

This test determines the ability of the isolate to hydrolyze tryptophan with the production of indole and pyruvic acid by the production of tryptophanase enzyme. The isolated organism was inoculated in tryptone broth. All the isolates were inoculated and incubated at 37℃ for 48 hrs. After incubation a reagent was used, added 5 drops of Kovac's reagent into the media Cherry red color appears at the top layer which indicates positive result no color changes show negative result.

Casein Hydrolysis Test:

This test determines the ability of isolates to break down milk protein casein by the activity of caseinase enzyme which hydrolysed casein into small amino acid. Skimmed milk agar was used for this test, the test isolates were incubated into the media and incubated at 37℃ for 24 hrs. clear zone shows positive result and no clear zone negative result.

Gelatin Hydrolysis Test:

This test was performed to determine the production of enzyme gelatinase which break down gelatin. Nutrient gelatine broth was used to inoculate the isolates and incubated at 37 for 24hrs or 48 hrs. and after 48 hrs. all the tubes were placed in refrigerator for 2 hrs. gelatine was still liquid shows positive test, as it hard shows negative result. This test is also known as liquefaction.

Nitrate reducing test:

Trypticase Nitrate broth was used for this test. Isolates were inoculated in this media at 37 for 24 hrs. Two reagents were used α-naphthylamine and sulfanilicacid were added to the media, these both compounds react with nitrite and turn red in color. The tubes were turn red because α-naphthylamine

and sulfanilic acid are already present in the tube. In few tubes nitrate was further reduced to ammonia or nitrogen gas. To distinguish between these two reactions, zinc dust was added. Zinc reduces nitrate to nitrite. The test organisms were able to reduce nitrate. Bright red color after the addition of α-naphthylamine and sulfanilic and no color change upon the addition of zinc was recorded as positive nitrate reduction test.

Antimicrobial activity against the Disinfectants

The isolates were again revived freshly for the test to get the best results. The nutrient broth was made and autoclaved at 121℃ for 15 mints at 15 psi. then it was allowed to cool down then the isolates were inoculated with the help of inoculating loop and incubated for 24hrs. hrs. at 37℃. Individual plates were prepared for each isolates as lawn cultures. Then the five most common disinfectants were selected that were diluted according to the required concentration **Dettol (10%), Savlon (10%), Ethanol (70%), Lysol (10%) and Hydrogen peroxide (3%).**The paper disc with 6mm diameter was selected and the dipped in the respective disinfected for a while then it was placed peripheral way and it was little pushed so that during the whole process the disc won't remove from their selected places in the petri plate which was previously prepared. Once the discs were placed the petri plates were again incubated at 37℃ for 8 hrs. after incubation the zone of inhibition was measured through a measuring scale. The area was zone of inhibition occurs become translucent or transparent which confirms the resistance or sensitivity for the disinfectants for certain bacteria/isolates.

RESULT AND DISCUSSION

The samples were collected from different age groups and male and females. The results of distribution of *Pseudomonas aeruginosa* isolates showed that *P. aeruginosa* associated with different patients' infections. There are different tests have been done for the identification of *P. aeruginosa*. Which includes morphological, biochemical, gram staining and the factors which effects the formation of biofilm. In present study it was found that the isolates may be *P. aeruginosa* on the basis of their characteristics according to the result.

Table 1: Morphology of Isolates on Nutrient Agar Media

Sl.No.	Isolates	Size	Pigmentation	Form	Margin	Elevation	Optical Character	Shape	Gram Stain
1	SHI-1	Large	Off-white	Irregular	Rough	Flat	Opaque	Short Rod	-ve
2	SHI-2	Large	Off-white	Irregular	Rough	Flat	Opaque	Short Rod	-ve
3	SHI-3	Moderate	Off-white	Irregular	Rough	Flat	Opaque	Short Rod	-ve
4	SHI-4	Pin Point	Off-white	Irregular	Rough	Flat	Opaque	Short Rod	-ve
5	SHI-5	Pin Point	Off-white	Irregular	Rough	Flat	Opaque	Short Rod	-ve
6	SHI-6	Large	Greenish	Rhizoid	Filamentous	Flat	Opaque	Short Rod	-ve
7	SHI-7	Large	white	Irregular	Rough	Flat	Opaque	Short Rod	-ve
8	SHI-8	Large	Greenish	Irregular	Rough	Flat	Opaque	Short Rod	-ve
9	SHI-9	Pin Point	Greenish	Irregular	Rough	Flat	Opaque	Short Rod	-ve
10	SHI-10	Large	Off-white	Irregular	Rough	Flat	Opaque	Short Rod	-ve

Morphology: The isolates were shows following morphological patterns.
+ve= positive,-ve = negative

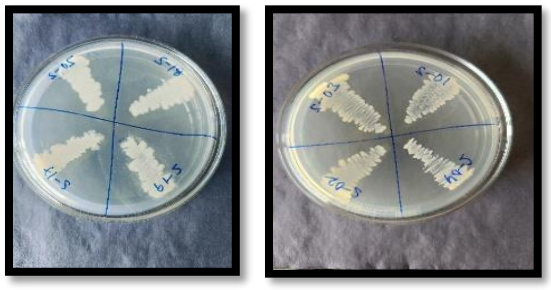

Fig1: Different isolated strains on Nutrient agar

Biochemical test: The isolates show different result after performing the biochemical tests. Biochemical test is second most important test in the field of bacteriology through which the identification and confirms isolates. In our finding isolates show positive Results for citrate, nitrate reduction, TSI test, Catalase, Oxidase and Mannitol and show negative result for test like MR, Indole, Urease, Lactose, Sucrose, VP etc. The biochemical test indicates that the isolates may be *pseudomonas aeruginosa* shown in Fig:2.

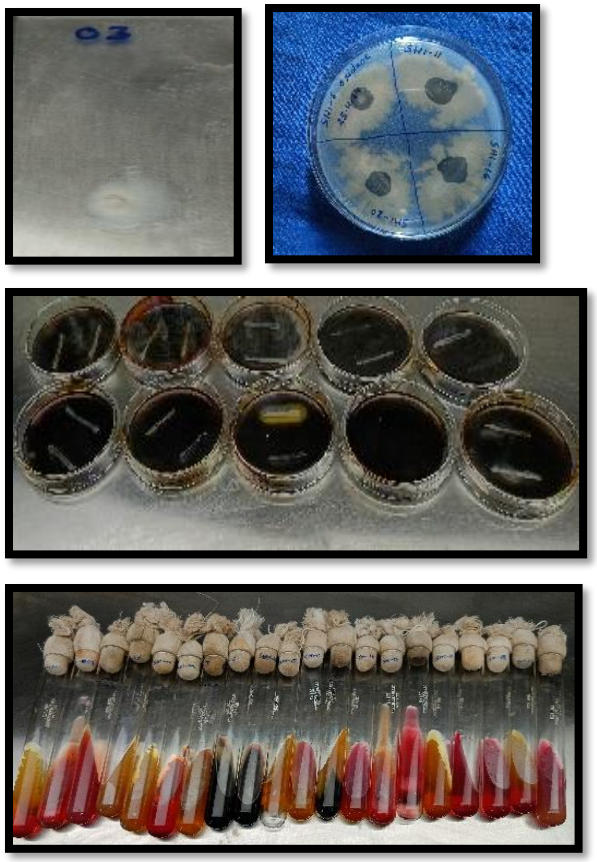

Fig 2: pseudomonas aeruginosa shows results for TSI

test, Catalase, Starch hydrolysis test and Oxidase Test

Triple sugar Iron reduction test:
The isolates show no change of color of butt of the tube. (Alkali/Alkali) (Red/Red)

Oxidase Test:
The isolates were positive as color changes to dark purple within 5- 10 sec and if it doesn't change it will be negative.

Catalase Test:
As the enzyme detoxifies hydrogen peroxide by breaking it into water and oxygen gas, the bubble formation indicates that the result was positive for catalase test.

Starch Hydrolysis test:
When Iodine was added to the isolates, they won't react with starch due to which no clear zone appeared. Which confirms that isolates may be pseudomonas aeruginosa

Sl. No	*Samples*	*Gram staining*	*Catalase test*	*Starch Hydrolysis*	*TSI test*	*Oxidase*	*Urease*	*M.R*	*VP*	*Indole*	*Casein*	*Gelatine*	*Nitrate Reducing test*	*Citrate*
1	*SHI-1*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*+ve*
2	*SHI-2*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*+ve*	*-ve*
3	*SHI-3*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*+ve*	*-ve*	*-ve*	*+ve*	*+ve*	*+ve*	*+ve*
4	*SHI-4*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*+ve*	*+ve*
5	*SHI-5*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*+ve*	*-ve*
6	*SHI-6*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*+ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*
7	*SHI-7*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*
8	*SHI-8*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*+ve*	*-ve*	*-ve*	*-ve*	*+ve*	*-ve*	*+ve*	*+ve*
9	*SHI-9*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*-ve*
10	*SHI-10*	*-ve*	*+ve*	*-ve*	*k/k*	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*+ve*

Table2 : Results for Biochemical Tests

Antimicrobial activity against Disinfectants:

In terms of sterilization and Disinfection the main motto was to reduce or kill the total pathogens. The disinfectants agents were mainly reducing the number bacteria on the surfaces. The pseudomonas aeruginosa itself one of the challenging bacteria in medical fields. All the disinfectants were selected on the basis of commercially and hospital used disinfectants. Disinfectants were used extensively in medical fields hospitals and other health care settings for a variety of topical and hard-surface applications (Olowe et al., 2004). It is an essential part of infection control practices and aid in the prevention of nosocomial infections (Rutala, 1995). The disinfectant reduces total the count of pathogenic organisms in a potential source of infection (Gerald and Denvar, 1999). Chemical agents used in disinfection are referred to as disinfectants and the three main types of disinfection available are cleaning, heating and disinfection with chemical agents (Geo et al., 2004). Most disinfectants are highly effective against pathogenic organisms and their effect can either be bacteriostatic or bactericidal (Ascenzi, 1996).The *P. aeruginosa*show the activity against the five selected disinfectants which were Dettol, Savlon, Lysol, Ethanol and Hydrogen Peroxide. There were several strains susceptible to the Savlon, Lysol and Dettol and some of the strain show resistance

toward the most common disinfectant that Ethanol and Hydrogen peroxide. Through our experiment it was also observed that the maximum strain of *P. aeruginosa* less susceptible toward the Dettol which is most common disinfectant among all the selected disinfectants, but most surprising result was for the Hydrogen peroxide and Ethanol which show high resistance toward them. Savlon and Lysol were high ranking in susceptibility. The result of this study clearly showed that most disinfectants are effective when used at correct concentrations. This is in agreement with the findings of Kaarina *et al*. (2000). Disinfectants like Savlon and Dettol were effective against many pathogenic organisms, especially when the number of cells present were not disinfected in the presence of excess **organic matter** a, it was observed by Olowe *et al*.
(2004). Out of five disinfectants the most effective was Lysol and Savlon after that Dettol showed some good susceptible result but disinfectants like ethanol and hydrogen peroxide showed very limited effect and maximum strains were highly resistance toward them.

Table 3: Different disinfectants and their criteria

Disinfectants	Resistant	Sensitive	Intermediate
Dettol	≤10mm	≥16mm	11mm and 15mm
Savlon	≤10mm	≥16mm	11mm and 15mm
Lysol	≤10mm	≥16mm	11mm and 15mm
Ethanol	≤10mm	≥16mm	11mm and 15mm
Hydrogen Peroxide	≤10mm	≥16mm	11mm and 15mm

Table 4: Effect of Different Disinfectant on *P.aeruginosa*

Sl. No	Samples	(Dettol) (10%) $A = \pi r^2$	Range (Diameter)	Antimicrobial sensitive test	(Savlon) (10%) $A = \pi r^2$	Range (Diameter)	Antimicrobial sensitive test	(Lysol) (10%) $A = \pi r^2$	Range (Diameter)	Antimicrobial sensitive test	(Ethanol) (70%) $A = \pi r^2$	Range (Diameter)	Antimicrobial sensitive test	Hydrogen Peroxide (3%) $A = \pi r^2$	Range (Diameter)	Antimicrobial sensitive test
1	SHI-1	13.67	24	S	12.64	26	S	13.36	28	S	0	0	R	4.60	11	R
2	SHI-2	0	0	R	11.61	24	S	11.87	25	S	0	0	R	4	10	R
3	SHI-3	5.19	12	I	11.10	23	S	13.16	27	S	0	0	R	0	0	R
4	SHI-4	5.19	12	I	13.67	28	S	10.06	21	S	0	0	R	4	10	R
5	SHI-5	5.19	12	I	4.60	11	I	9.01	19	S	0	0	R	1.80	7	R
6	SHI-6	2.64	8	R	4	10	R	8.48	18	S	0	0	R	0	0	R
7	SHI-7	0	0	R	0	0	R	0	0	R	0	0	R	0	0	R
8	SHI-8	5.19	12	I	12.64	26	S	11.61	24	S	0	0	R	0	0	R
9	SHI-9	7.41	16	S	10.06	21	S	9.01	19	S	7	1.80	R	8/2.64	8	R
10	SHI-10	9.53	20	S	7.95	17	S	7.95	17	S	7	1.80	R	0	0	R

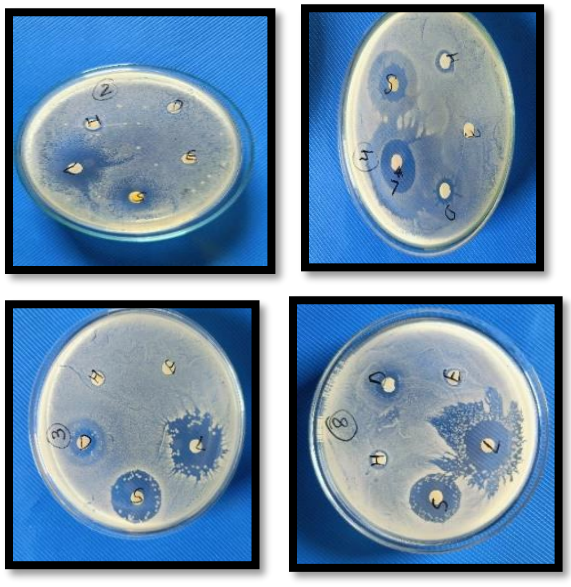

Fig 3: Effect of different disinfectants on *p. aeruginosa*

CONCLUSION

From the above experiments it was observed that the *pseudomonas aeruginosa* highly resistance against Dettol, Hydrogen peroxide and Ethanol which were most commercial and clinically used disinfectants. In our experiments it was observed that these isolates become sensitive towards **Savlon, Lysol** and few shows intermediate results. The result concluded that long uses of such disinfectants destroyed the pathogens but after some time they become resistant toward the disinfectants. The ingredients of the disinfectants were different which show different impacts. The disinfectants which are commonly used for the eradication of the *P.aeruginosa* we did an experiment to know the quality and quantity of the reaction against the disinfectants. The pathogenicity mechanisms of *P. aeruginosa* biofilms, including their characteristics and

QS properties, are extremely complex. Although the structure of a biofilm seems to be simple, the genes and mechanisms involved in biofilm formation are diverse and hard to comprehend. Furthermore, most of the studies to identify them have been conducted in vitro, which is not the real situation in human infections. The use of transcriptomics and metabolomics to analyse clinical sample could be an approach to address this.

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