RESEARCH ARTICLE

Antibiotics Susceptibility of Environmental Bacteria: *Ralstonia pickettii* and *Pseudomonas luteola* Isolated from Shatt Al-Hilla in Babylon Province, Iraq

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Received: 20th September, 2020; Revised: 09th October, 2020; Accepted: 18th November, 2020; Available Online: 25th March, 2021

ABSTRACT

Introduction: *Ralstonia pickettii and Pseudomonas luteola* are opportunistic pathogens; gram-negative bacteria have been found in moist soil which causes pollution of pure water which leads to many diseases by these pathogens.

Results: In the current study, *R. pickettii* and *P. luteola* were isolated from the Shat Al-Hilla in Babylon province. In this study were found *R. pickettii* more resistant to antibiotics than *P. luteola* and not sensitive to any antibiotics that used in the test while intermediate to four antibiotics from seventeen antibiotics. Whilst *P. luteola* sensitive to three antibiotics include Gentamicin, Ciprofloxacin, Trimethoprim/Sulfamethoxa, and resistance to seven antibiotics.

Conclusion: It has been concluded that *R. pickettii* more resistant to antibiotics and do not have an sensitive to any antibiotic used in this study compared to *P. luteola* that proved sensitive to some antibiotics.

Keywords: Antibiotic susceptibility, Pollution, *P. luteola*, Opportunistic pathogen, *Ralstonia pickettii,* Vitek.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.1.32

How to cite this article: Mohammed HA, Muttalib Al-Shalah LA, Althahab AO. Antibiotics Susceptibility of Environmental Bacteria: *Ralstonia pickettii* and *Pseudomonas luteola* Isolated from Shatt Al-Hilla in Babylon Province, Iraq. International Journal of Drug Delivery Technology. 2021;11(1):175-179.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

It has been renamed *R. pickettii* twice in its history since isolated for the first time clinical specimens and renamed in 1973.[1] It was located originally with *Pseudomonas* family, then *Burkholderia*. [2] divided *Ralstonia* spp. known to date into two lineages, Ralstonia and Wautersia. The *Ralstonia pickettii* lineage include; *R. pickettii*, *R. insidiosa*, *R. mannitolilytica*, *R. syzygii*, *R. solanacearum*. *Ralstonia* is a recently specified group that included previous components of *Burkholderia* species *Burkholderia pickettii*, *Burkholderia solanacearum*, and recalled as *R. pickettii* and *R. solanacearum*.[3] *R. pickettii* is respiratory gram-negative, oxidase-positive, no fermentative rod and is all over the place micro-organism found in water and soil.[4] *R. pickettii* has been caused a biofilm formation in plastic water pipes.[5] *R. pickettii* has been founded in high purity water in industrial systems,[6] in the Space Shuttle water system,[7] and in laboratory-based purified water devices.[8]

It has the capacity to remain alive in the little nourishing conditions,[9] that is believed in high purity water systems, the bacteria may be able to eliminate from the plastic polymers in the tubing. *R. pickettii* does not represent pathogen bacteria. However, she was isolated from patients with cystic fibrosis.[10,11] Virulence for this bacteria is low, but it is associated with pseudo bacteremia.[12-14] It has been segregated from unusual clinical situations, including osteomyelitis,[15] septic arthritis[16] and invasive infections in drug users.[17] It has been related to continuing indwelling intravenous devices,[18] where four cases were discovered with a wide temporal distribution (2 years), suggesting that contaminated fluids were not involved and was not determined the bacteria's origin. In comparison, the contaminating fluids were related to a series of Hickman catheter-associated *P. pickettii* infections over a 3 months period in a pediatric oncology unit in S. Africa, when seven patients grew septicemias. The source of the contamination was attributed to sterile distilled water.[19] *R. pickettii* has shown itself to be flexible to remediation in water stocks with impedance to disinfectants such as chlorhexidine; the bacterium has been shown to permeate 0.2-micron filters.[20-22]

P. luteola is an opportunistic pathogen in patients with implied medical trouble or with lodging catheters; cases of septicemia, septic arthritis, meningitis, endocarditis, and peritonitis with this uncommon bacterium were reported. It is

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located everywhere in humid habitats,at first specified in the genus *Chryseomonas*, the species has been transported to the genus *Pseudomonas*. *P. luteola* is a gram-negative aerobe, by used multi-trichous flagella for movement.[23] An environment in which *P. luteola* are present is not specific, but most often live in a humid environment, and develop as rods of 0.8 - 2.5 µm.[24] They can polluted solutions such as distilled water, disinfectants, and intravenous solutions.[25] In a few and rare cases, *P. luteola* have been registered as a pathogen.[26] *P. luteola* lead to the blood circulating pollutions linked together inside vascular, indwelling catheters, pancreatitis, prosthetic valve endocarditis, alien bodies and cutaneous ulcer,[27] and an important lead to healthcare-correlating pollutions, especially among infants in neonatal intensive care units (NICUs), Some information on the presence of *Pseudomonas* spp in NICUs was noted.[28] The current study aims to isolate and diagnose *R. pickettii and P. luteola* from river water because it is one of the opportunistic bacteria and causes pollution of water pipes and tanks and knowledge of antibiotics used to eliminate it.

METHODS

Specimens Assembly

From Shatt Al-Hillah, 65 specimens were gathered. In sterile glass vessels, water samples were placed Then, the water samples were cultured onto nutrient agar by transport 0.1 mL from water samples to the Petri dish plate.[29]

Isolation of Suspected *R. pickettii* and *P. luteola*

Suspected isolates of *R. pickettii* and *P. luteola* cultivated on nutrient agar; isolates were grown aerobically and incubated overnight at 37°C.[30] Suspected isolates of *R. pickettii* and *P. luteola* cultivated on MacConkey agar.[31]

Biochemical Diagnostic Kit (Vitek system).

For diagnostic bacterial isolates are listed in Table 1 by used Vitek kits.

Recognition by utilizing VITEK 2 Fluorescent Apparatus

VITEK 2 DensiCheck instrument,fluorescence apparatus (bioMe´rieux) (ID-GNB card) consist of 43 non-intestinal gram-negative ranking. On the report of the guidance of the industry, a trial was completed. Isolates were cultured on LB agar at 37°C for 18 to 24 hours before the isolate was subjected to analysis, adjusted of stuck bacterial to a McFarland standard of 0.50–0.63 in a blend of 0.45% sodium chloride utilizing the VITEK 2 tool. The period among the preparation of the mixture and the loading of the card was every time less than one hour. The recognition card for gram-negative bacteria with 41 fluorescent biochemical trials for investigation every 15 minutes was read the cards automatically utilizing the VITEK 2 software version VT2- R03.1 to examined information.[32]

Table 1: Diagnostic Kit with its Manufactured company.

Purpose	*Kit*	*Industry company (Origin)*
Gram-negative Identification	VITEK®2 GN	Biomerieux (France)
Antibiotic Susceptibility	VITEK AST	

Sensitivity Testing for Antibiotics

The basic process was utilized to test the sensitivity of *R. pickettii, and P. luteola* to some antibiotics, including ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, minocycline, colistin, trimethoprim/sulfamethoxazole.[33] For environmental isolates of *R. pickettii and P. luteola* utilized VITEK 2 DensiCheck device (bioMe´rieux). In Table 2 shown antibiotics and their abbreviation.

RESULTS

From 65 specimens, only 7 (10.76%) samples were identified as the suspected *R. pickettii,* and 5 (7.69%) samples were identified as the suspected *P. luteola*. The suspected isolates were diagnosed by VITEK 2 DensiCheck apparatus. The results showed two isolates (3.07%) for *R. pickettii*, Whilst one isolate (1.53%) for *P. luteola* (Table 1). That is why it is used to distinguish *R. pickettii* and *P. luteola's* environmental isolates in the current study.

The suspected *R. pickettii* and *P. luteola* isolates were identified according to color of colony that produced acid from glucose oxidatively and produce yellow pigment onto nutrient agar and MacConkey agar.[31] In addition, VITEK 2 was used to determine *R. pickettii* and *P. luteola* because this technique is high accuracy and very rapid compared to other techniques (such as biochemical method). Various studies used VITEK technique for diagnosing the isolates.[34] VITEK 2 DensiCheck device technology was used to determine the sensibility and rigid of clinical and environmental isolates to a broad range of antibiotics. In the present study, the MIC of each antibiotic was measured against twelve environmental isolates of *R. pickettii and P. luteola*. In the Table 2 showed the MIC of Seventeen antibiotics TIC, TIC/CLA, PIP, PIP/TAZ, CEF, CEF, AZT, IMI,

Table 2: Antibiotics utilizing in sensitivity testing by VITEK 2.

No.	*Antibiotics*
1	Ticarcillin
2	Ticarcillin/Clavulanic acid
3	Piperacillin
4	Piperacillin/Tazobactam
5	Ceftazidime
6	Cefepime
7	Aztreonam
8	Imipenem
9	Meropenem
10	Amikacin
11	Gentamicin
12	Tobramycin
13	Ciprofloxacin
14	Minocycline
15	Colistin
16	Trimethoprim/Sulfamethoxazole

Table 1: Number and percentage of *R. pickettii* and *P. luteola* isolated from environmental samples.

No. of samples	*Isolated sample*	*No. of suspected isolates*	*Percentage suspected isolates (%)*	*Isolates diagnosis by VITEK2*	*Percentage (%)*
65	*R. pickettii*	7	10.76	2	3.07
	P. luteola	5	7.69	1	1.53

Table 2: Antibiotics susceptibility and MIC of various antibiotics tested against environmental isolates of *R. pickettii* that collected from Shatt al-Hillah, by utilized VITEK 2 DensiCheck tool for minimum inhibition concentrations MIC testing of antibiotics.

Antibiotics	*MIC*	*Susceptibility information*
TIC	>64	R
TIC/CLA	64	I
PIP	>64	R
PIP/TAZ	>64	R
CEF	>32	R
CEFE	32	R
AZT	>32	R
IMI	>8	R
MER	8	I
AMI	>32	R
GEN	>8	R
TOB	>8	R
CIP	2	I
MIN	8	I
COL	>8	R
TRI\ SUL	80	R

Table 3: Antibiotics susceptibility and MIC of various antibiotics tested against environmental isolates of *P. luteola* that collected from Shatt al-Hillah, by utilized VITEK 2 DensiCheck tool for minimum inhibition concentrations MIC testing of antibiotics.

Antibiotics	*MIC*	*Susceptibility information*
TIC	>64	R
TIC/CLA	64	I
PIP	64	I
PIP/TAZ	>64	R
CEF	>32	R
CEFE	16	I
IMI	8	I
MER	>8	R
AMI	>32	R
GEN	< =1	S
TOB	>8	R
CIP	0.5	S
MIN	>8	R
TRI\ SUL	< =20	S

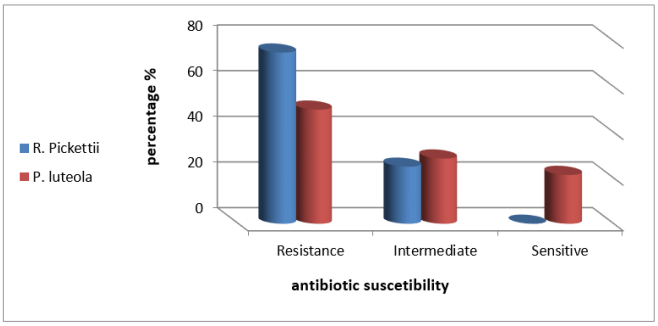

Figure 1: The percentage of antibiotics susceptibility in *R. pickettii* and *P. luteola* isolates.

MER, AMI, GEN, TOB, CIP, MIN, COL, TRI/SUL against environmental isolates.

The results showed that from 65 samples, three environmental isolates (1-isolate of *R. pickettii* and two isolates of *P. luteola*) were resistant to most antibiotics that were used in the current study.

DISCUSSION

R. pickettii is a nosocomial communicable agent and an important industrial contaminant. The bacterium is found in many various environments, including clinical cases, soil and manufacturing high purity water.[35,36] It was famous as an occasional factor of contagion of skeleton and joints,lead to infective endocarditis, severe pneumonia, and fulminant sepsis.[37-41] *R. pickettii* was resistant to (12 antibiotics) include TIC, PIP, PIP/TAZ, CEF, CEFE, AZT, IMI, AMI, GEN, TOB, COL, TRI/SUL, but intermediate to(4 antibiotics) include TIC/CLA, MER, CIP, MIN, and not susceptible to any antibiotic. While *P. luteola* was resistance to (7 antibiotics) include TIC,PIP/TAZ,CEF,MER,AMI,TOB,MIN, while intermediate to (4 antibiotics) include TIC/CLA,PIP,CEFE,IMI and sensitive to (3 antibiotics) include GEN,CIP,TRI/SUL. The highest minimum inhibition concentrations (MIC) of TRI/SUL antibiotics about 80 and *R. pickettii* resistance to these antibiotics while other studies[35] explained most isolates of *R.picketti* sensitive to TRI/SUL are shown in Table 2. In contrast, other studies appeared *R. pickettii* susceptible to CIP and TRI\SUL.[36] In the present study was showed the isolate of *R. pickettii* resistance to ceftazidime, while other researchers showed the bacterium sensitivity to these antibiotics.[35]

R. pickettii showed resistance to the imipenem antibiotic, and this is consistent with other research has shown that for various antimicrobial agents *Ralstonia* species it was solid, mostly β-lactams (inclusive carbapenems) presented existence of the group D β-lactamases bla_{OXA-22} and bla_{OXA-60} genes, the close amino acid symmetry of OXA-444 protein to the OXA-60, and the carbapenem strength included imipenem that illustrates the existence of the serin-hydrolase class C beta-lactamase.[36] These enzymes, linked with various mechanisms such as above expression of the flowing out of a particular substance or particle forces, that lead to resistance to different types of antibiotics.[37]

P. luteola is one of the no fluorescent groups of the family Pseudomonadaceae. The bacterium is an environmental organism.[26] and scarcely causes community- or hospital-acquired infection in humans.[38] In the current study, antibiotic sensitivity assay evidenced that *P. luteola* resistance to TIC,PIP/TAZ,CEF,TOB,MIN, AMI and MER antibiotics are shown in Table 3, while[26] were reported in their study that AMI, GEN, TRI\SUL, and MER had effective activity against *P. luteola* isolates clinical isolates and most strains were resistant to some of the antipseudomonal antibiotics. In the present study were registered *P. luteola* was sensitive to GEN, CIP, TRI/SUL while Brady MT, *et al*.,[26] were reported that *P. luteola* isolates were sensitive to IMI, CIP, and CEF. *R. pickettii* more resistant to antibiotics than *P. luteola* that appeared sensitive to more antibiotics, the percentage of antibiotics resistance in *R. pickettii* about 75%, intermediate 25%, and not sensitive to any antibiotics. whilst in *P. luteola* about 50% (resistance), 28.57% (intermediate), 21.4% (sensitive) (Figure 1).

CONCLUSIONS

In the current study, The suspected *R. pickettii* and *P. luteola* isolates were identified according to color of colony that produced acid from glucose oxidatively and produce yellow pigment onto nutrient agar, and MacConkey agar, *R. pickettii* and *P. luteola were diagnosed by* VITEK 2. Three environmental isolates (1 isolates of R. pickettii and 2 isolates of P. luteola) were resistant to most antibiotics used in current study from 65 samples. *R. pickettii* was resistant to 12 antibiotics, but intermediate to 4 antibiotics, and not susceptible to any antibiotic. While *P. luteola* was resistance to 7 antibiotics, while medium to 4 antibiotics and sensitive to 3 antibiotics. *R. pickettii* more resistant to antibiotics and does not have a sensitivity to any antibiotic used in this study compared to *P. luteola* that proved sensitive to some antibiotics. The results can give more insight to researchers interested in this field.

ABBREVIATIONS

R. pickettii: *Ralstonia pickettii*; *P. luteola*: *Pseudomonas luteola*; NICUs: neonatal intensive care units; LB agar: Luria-Bertani agar; Ticarcillin: TIC; Ticarcillin\Clavulanic acid: TIC\CLA; Piperacillin: PIP; Piperacillin/Tazobactam: PIP\TAZ; Ceftazidime: CEF; Cefepime: CEFE; Aztreonam: AZT; Imipenem: IMI; Meropenem: MER; Amikacin: AMI; Gentamicin: GEN; Tobramycin:TOB; Ciprofloxacin:CIP; Minocycline: MIN, Colistin: COL; Trimethoprim/Sulfamethoxazole: TRI\SUL; S: susceptible for an antibiotic, R: resistance, I: Intermediate.

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