Study on controlling the action of Plant extracts of *Terminalia bellirica, Aegle marmelos* and *Adhatodavasica* against selected uropathogens

[a]Vinita Turkar, [b]Arun Kumar, [c]Prabhakar Bhandari

[a]Research scholar, Department of Microbiology, JJT University, Vidyanagari, Jhunjhunun, Rajasthan, India
[b]Professor, Department of Microbiology, JJT University, Vidyanagari, Jhunjhunun, Rajasthan, India
[c]Assistant Professor, Department of Microbiology, Sevadal Mahila Mahavidyalaya, Nagpur, India

Corresponding Author: Vinita Turkar

Abstract

Urinary tract infection is certainly on rising due to bacterial infection and also possessing multidrug-resistant features and that demands plant-based remedy. In the present study, three plants *Terminalia bellirica, Aegle marmelos* and *Adhatodavasica* extracts prepared in the number of solvents and tested against Uropathogens *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Salmonella enterica* isolated earlier. Result showcased that all plants represent its antibacterial feature with the given solvent preparation against particular pathogens either alone or it also minimizes antibiotic resistance when working in synergy. In conclusion, it could be put forward that UTI may be possible to control by using these plant preparation which is promising and put forward its commercial application in coming time to control MDR uropathogens.

KEYWORDS: Urinary tract infection, antibiotic resistance, Plant extract, Synergistic activity.

Introduction:

Increasing reports of multidrug resistance put up the sever problem in front of us. As this resistance demands economic burden on the consumer as estimated to be 20 billion dollars per year in the US only (Cosgrove, 2006; DiazGranados et al., 2005; Sydnor and Perl, 2011). Not only is that as per estimation until 2050, with the exposure to antibiotic-resistant microbe's estimated 3000 premature deaths could be possible (AMR, 2014). This situation certainly will increase as new antibiotics are continue to get introduced into the market. The severity of antibiotics further increases when both Gram positive and Gram negative reported with Drug resistance when those become resistant to more than the antimicrobial agent (Anderson et al., 2006; Cosgrove et al., 2003; Roberts et al., 2009).

In a remedy, plant-based products have surely been successful to control MDR in an environment since plant-based content interact to affect the protein-protein interaction in surely brings about the change in cellular signalling (Koehn and Carter, 2005). Plant content acts by secondary metabolites to bring about the interruption of DNA/RNA synthesis and function (Anandhi et al., 2014); disruption of function and efflux system (Sanchez et al., 2010) and inhibition of quorum sensing (Nazzaro et al., 2013).

Now nearly every person once in a lifetime suffers from urinary tract infection which represents features like frequent and painful urination, feeling to do urgent urination and supra-pubic pressure (Foxman et al., 2000). UTI is more server with patients having bladder catheterization (Platt et al., 1982); also, in children (Loening-Baucke, 1997) and in the sexually active woman (Hooton et al., 1996). In a remedy, the present study investigated plants-based extract capable to control multidrug-resistant pathogens isolated from urinary tract infection by using them alone or in synergistic mode.

Material and Methods

Isolation of bacterial pathogens from urine:

Patients reported with urinary tract infection and visited local laboratories were carefully selected and their urine samples were collected in sterile vials as per routine lab protocol.

Plating on selective media:

Presence of Urine samples with *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Salmonella enterica* intensely targeted in the urine samples and confirmed by inoculating urine (100µl) on the Pseudomonas isolation agar, MacConkey agar and Eosin methylene blue agar, respectively. Further plates were incubated at 37 for 24 hours and appeared colonies were confirmed for the concernedspecies and those were Gram stained accordingly.

Identification of isolates by 16S rRNA gene:

Even though medium such as *Pseudomonas isolation agar, Eosin methylene blue agar,* and *MacConkey agar* allows selective growth of isolates as *P. aeruginosa, K. pneumoniae,* and *S. enterica*. They were further identified by targeting the 16S rRNA gene as per the protocol followed by Rai et al., (2013).

Plants as an effective antimicrobial agent:

In the present study three plants identified as *A. acanthaceae, A. marmelos* and *T. bellerica* were produced from the local regions of Gondia. These plants were processed for plant extract preparation by involving petroleum ether, methanol, ethanol and distilled water. Here 30ml of solvent was added with 10 grams of plant part and kept on incubating in dark for the next 96 hours. Upon incubation, content was filtered using a muslin cloth and the filtrate was directly kept on evaporating in a Petri-plate when the temperature set at 90. Here obtained concentration upon evaporation was re-dissolved in 2ml of 0.9% DMSO and used thereafter.

Antibiotic sensitivity check:

Identified isolates such as *P. aeruginosa, K. pneumoniae* and *S. enterica* were screened for their available antibiotic sensitivity when tested by Kirby Bauer Disc diffusion method. Here 16 antibiotics were screened such as Polymyxin B (PB300), Imipenem (IPM10), Norfloxacin (NX10), Ceftriaxone (CTR30), Cefoperazone (CPZ75), Piperacillin/ Tazobactam (PIT 100/10), Mezlocillin (MZ 75), Aztreonam (AT30) Netillin (Net 30), Carbenicillin (CB100), Meropenem (MRP10), Ceftazidime (CAZ 30), Ceftizoxime (CZX30), Gatifloxacin (GAT 5), Gentamycin (GEN 10), and Tobramycin (TOB 10). Inoculated plates were incubated at 37temperature for 24

hours and obtained zone of inhibition was measured in millimetre as per CLSI chart and strains were classified as sensitive, intermediate and resistant.

Plant extract antibacterial activity:

In a process prepared plant extract antibacterial activity was tested by well diffusion assay. Isolates were inoculated on solidified nutrient agar plates set at 1 O.D. with the volume of 100µl.The medium was then punctured with wells which were loaded with 100µl of plant extract prepared in DMSO. These plates were kept steady for 45 minutes in an order to obtain complete diffusion of extract. All plates were incubated at 37 for 24 hours. Upon incubation, the developed zone of inhibition was recorded in millimetre.

Synergistic activity of antibiotics and plant extract:

Based on the previous study, promising plant extract was used in a synergistic activity with those antibiotics recorded to be resistant by the strains. Here molten nutrient agar mediumwhen set (15ml) at 45-50 was firstly mixed with 100µl of plant extract and mixed well. Later on, it was allowed to form a solidified layer. On this layer, MDR isolates of *P. aeruginosa, K. pneumoniae,* and *S. enterica* was individually inoculated by spread plate method. All plates were kept steady for 20 minutes and then inoculated with antibiotics discs. These plates were then incubated for 24 hours at 37 temperature. Results were recorded as a zone of inhibition by referring CLSI chart and improved sensitivity was recorded with the effect of synergy.

Result:

Bacterial species isolation:

Urine samples when inoculated on the selective media total 10 isolates each of *P. aeruginosa, K. pneumoniae,* and *S. enterica* was confirmed to be present when detected on Pseudomonas isolation agar,MacConkey agar and Eosin methylene blue agar as in Fig. 1. Upon Gram staining, *P. aeruginosa* and *K. pneumoniae* as Gram negative rod whereas *S. enterica* stained as Gram-negative rod as in Fig. 2.

Antibiotic sensitivity:

With 16 antibiotics tested for their activity against *K. pneumoniae, P. aeruginosa,* and *S. enterica* following results were obtained.

Here in isolate *P. aeruginosa* (n=10) most sensitive nature was recorded with Tobramycin, Gentamicin and Piperacillin.Ceftizoxime recorded the most resistant (40%) followed by Polymyxin B and Ceftriaxone (30%); 20% resistant obtained in Cefoperazone, Mezlocillin, Aztreonam, Netillin and Ceftazidime with and lastly10% strains registered resistance to Imipenem, Norfloxacin, Meropenem and Gatifloxacin as in Table 1 and Fig. 3.

Isolate *S. enterica* (n=10) recorded higher resistance to antibiotic Polymyxin B (60%) followed by Carbenicillin, Gentamicin, Tobramycin and Cefoperazone (40%); Norfloxacin (30%); Ceftriaxone, Mezlocillin, Meropenem, Aztreonam with 20% strain resistant. In a controlling action, antibiotic Gatifloxacin remained the most effective by showing a high level of sensitivity Table 2 and Fig. 4.

Isolate *K. pneumoniae* (n=10) registered highest resistant towards Ceftizoxime (70%) followed by Polymyxin B, Norfloxacin, Meropenem, Ceftazidime and Aztreonam

40%; Carbenicillin and Cefoperazonerecorded with 30% resistance, Ceftriaxone, Mezlocillin, Netillin, Gatifloxacinand Gentamicin with 20% and least resistance recorded with Tobramycin as in Table 3 and Fig. 5.

Antibacterial activity of Plant extract:

As per well diffusion assay, Plant *A. vasica* bark extract capable to inhibit *P. aeruginosa* with extracts prepared in petroleum ether, ethanol, methanol as well as aqueous solvents mode with 10, 10, 11 and 10mm of the zone of inhibition, respectively. Here *K. pneumoniae* and *S. enterica* did not showcase any sensitivity.*A. vasica* leaf extract capable to control *S. enterica* with ethanol extract (12mm) but *P. aeruginosa* and *K. pneumoniae* remained non-responsive to sensitivity (Table 4).

Plant *A. marmelos* bark extract recorded better zone of inhibition with *S. enterica* (17mm) in aqueous extract followed by ethanolic extract (15mm). *P. aeruginosa* also remained sensitive to aqueous extract (14mm) and then by Petroleum ether, ethanol and methanol (13mm). Here *K. pneumoniae* remained resistant for every plant extract (Table 4). Plant *A. marmelos* leaf extract capable to inhibit *P. aeruginosa* the most (15mm) with ethanolic and methanolic extract. Here all other isolates remained resistant (Table 4).

Plant *T. bellerica* bark extracts remained the most effective to control *S. enterica* with methanol, ethanol, and aqueous extract recorded with 23mm, 20mm, and 19mm of inhibition, respectively. All other species remained resistant in this set (Table 4).

Plant *T. bellerica* leaves extracts recorded to control *P. aeruginosa* growth with the extracts of ethanol and methanol having 20 and 21mm of inhibition, respectively while others remained resistant (Table 4).

In *T. bellirica*fruit set *S. enterica* remained highly sensitive with ethanolic, methanolic and aqueous extract with 32mm, 31mm, and 30mm of the zone of inhibition respectively. *P. aeruginosa* got inhibited by ethanolic, methanolic and aqueous extract with 15mm, 14mm and 16mm of inhibition. *K. pneumoniae* also registered its sensitivity with ethanol, methanol and aqueous extract with 13mm, 12mm, and 14mm of inhibition, respectively as given in Table 4.

Synergistic activity of plant extract with Antibiotics:

The overall study put forward that *T. bellirica*(Behada), *A. marmelos* and *A. vasica* able to showcase better antibacterial activity, further study was carried out with antibiotics in a synergistic assay. In testing against *Pseudomonas aeruginosa* Bel bark petroleum ether extract able to give increased sensitivity with antibiotics Imipenem (13mm); Aztreonam (23mm); Ceftazidime (10mm); and Ceftizoxime (19mm).

In an ethanolic extract, Bel bark able to inhibit *P. aeruginosa* as 27mm with Aztreonam followed by Ceftazidime with 19mm of inhibition.

In the case of bale barkmethanol extract, Aztreonam found to be most effective against *P. aeruginosa* with 29mm of inhibition.

In a similar study, Bel ethanolic leaves extract with aztreonam (21mm); Behada leaf extract with Ceftizoxime (23mm); Behada fruit extract in ethanol with Ceftizoxime (14mm); Beheda methanolic extract of fruit (10mm) and Beheda aqueous extract of fruit with 25mm of inhibition was recorded with *P. aeruginosa.*

Similarly in plant Adulsa (*A. vasica*) higher zone of inhibition with *P. aeruginosa* was recorded in bark petroleum ether in synergy with Aztreonam when tested with *P. aeruginosa* (Table 5).

In the case of *K. pneumoniae,* sensitivity recorded to increase what tested with *T. bellerica* (Behada). Here Behada fruit ethanolic extract registered higher inhibition with Carbenicillin (32mm).

In methanolic and aqueous extract as such minimum inhibition of growth was recorded as 12mm and up to 19mm which indicated a low synthesis of bio-compounds as compared to fruit ethanolic extract (Table 5).

In a set of *S. enterica* Bel bark petroleum extract significantly improved sensitivity in the presence of Ceftizoxime (30mm) which was at least two-fold higher than only antibiotics treatment.

In the case of Behada bark ethanolic extract, Ceftizoxime was recorded to be inhibiting the growth of *S. enterica* as 26mm and in methanollic extract with Imipenem it was 24mm. Along with these extract the most promising plant extract recognised as aqueous bark extract of Behada along with Aztreonam able to inhibit up to 36mm which was highest up to 36mm which was highest among all sets as in Table 5.

16S rRNA gene sequencing:

Lastly, MDR *P. aeruginosa, K. pneumoniae,* and *S. enterica* were identified successfully by targeting their 16S rRNA gene and based on BLASTN homology and phylogenetic analysis up to species level these isolates were identified as in Fig. 6, 7, and 8.

Discussion:

Present study successfully reported the presence of *P. aeruginosa, K. pneumoniae* and *S. enterica* in the urine sample of UTI patients. They were isolated on selective media such as Pseudomonas isolation agar (*P. aeruginosa*), MacConkey agar (*K. pneumoniae*) and Eosin methylene blue agar (*S. enterica*) successfully. Many such have published to freshly isolate these organisms from urine and another source by using these media similar to the present study (Álvarez-Otero et al., 2017; Bachiri et al., 2017; Goodman et al., 2017). It is put forward that *P. aeruginosa* registered about 40% resistance towards Ceftizoxime and 30% resistance by strains towards Polymyxin B and Ceftriaxone. Here it is also been recorded that ≥40% strains were MDR (n≥3) for the urine samples. In some source of isolation, the presence of MDR *P. aeruginosa* was reported earlier also (Alvarez-Otero et al., 2017; Pereira et al., 2015; Fusté et al., 2013). Here *S. enterica* also registered with 60% resistance towards Polymyxin B, 40% with Cefoperazone, Carbenicillin, Gentamycin and Tobramycin. In a similar study, Kwambana-Adams et al., (2015) reported MDR *S. enterica* in the urine sample with 14% (21/152) prevalence. Similarly, Ranjbar et al., (2017) reported *S. enterica Serovar Typhimurium* presence in urine sample having 80% population MDR features with ≥3 antibiotics.

Present study highlighted that this multidrug resistance *P. aeruginosa, K. pneumoniae* and *S. enterica* possible to control when single or synergistic treatment of plant extracts with or without been given. In many reports success of plant *T. bellerica, A. marmelos* and *A. vasica* towards antibacterial activity have been given which was

confirmed once again when tested against uropathogens. According to Jindal et al., (2013) *A. marmelos* fruit pectin showcase antimicrobial activity and hence remain commercially important. *T. bellerica* with an alcoholic extract able to showcase antibacterial activity against *S. aureus* and *S. pyogenes* (Kumar et al., 2008). *A. vasica* extract also reported to be positive for antibacterial activity due to the presence of vasicine, vasicinone, and vasicine acetate and able to inhibit *E. coli* at 755µg/ml concentration. A similar report of *A. vasica* and its antibacterial features reported by Duraipandiyan et al., (2015); Ignacimuthu and Shanmugam, (2010).

Conclusion:

Plant-based extracts of *T. bellerica, A. marmelos,* and *A. vasica* certainly been useful to control the growth of *P. aeruginosa, K. pneumoniae* and *S. enterica* isolated as uropathogens. This feature could be represented by plant extract direct action and/or in synergy with antibiotics so that future antibiotic resistant could be controlled.

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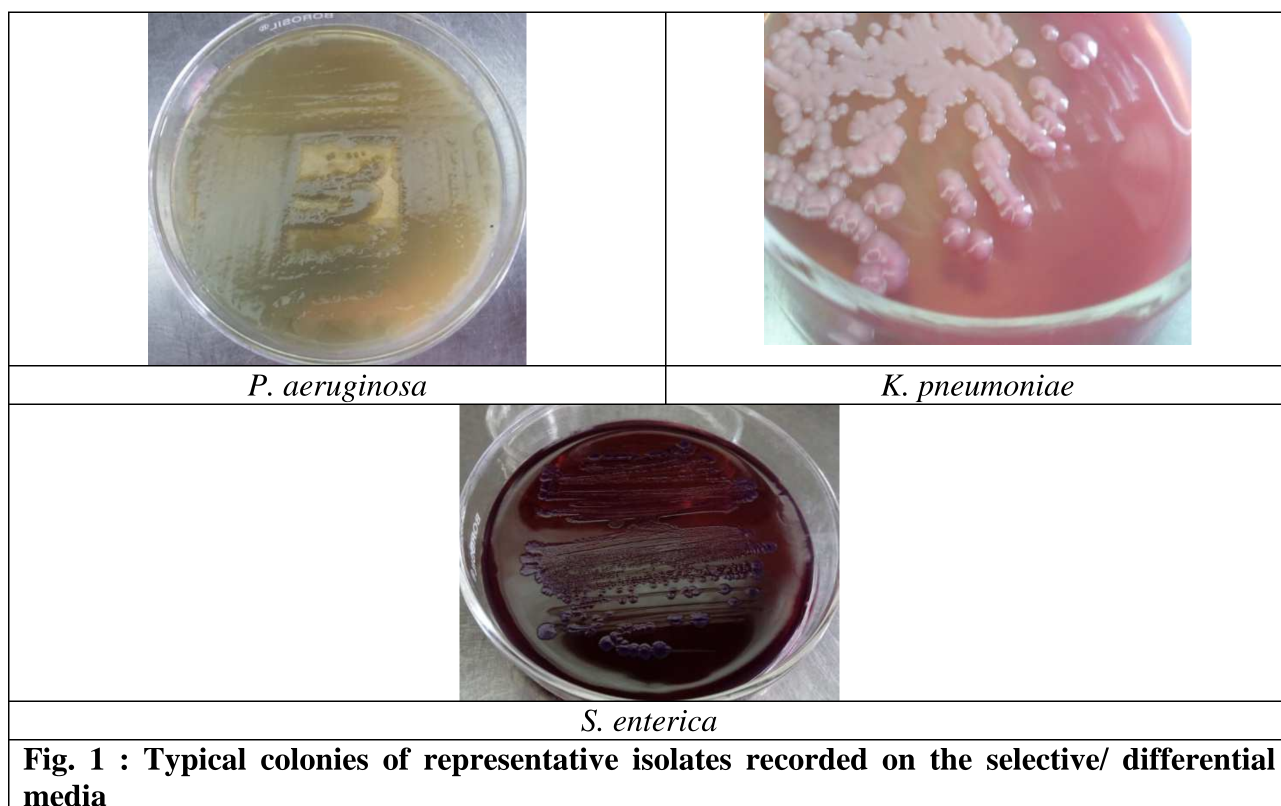
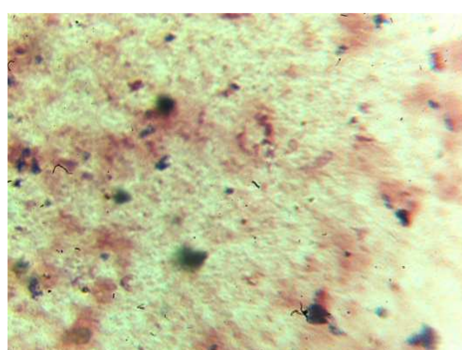


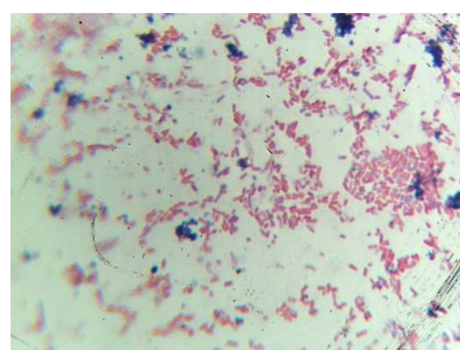
P. aeruginosa | *K. pneumoniae*

S. enterica

Fig. 1 : Typical colonies of representative isolates recorded on the selective/ differential media

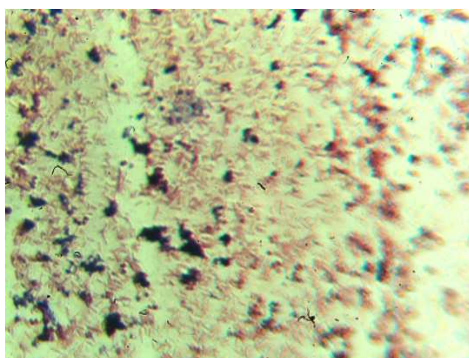


P. aeruginosa



K. pneumoniae

Fig. 2: Micrograph of Gram staining captured at 100X magnification



S. enterica

Table 1: Antibiotic sensitivity against *P. aeruginosa* strains

Antibiotics	abbreviations	Zone of Inhibition (mm)									
		P. aeruginosa									
Strains		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Polymixin-B	**PB300**	S	S	S	S	R	S	R	S	S	R
Imipenem	**IPM10**	S	R	S	S	S	S	S	S	S	S
Norfloxacin	**NX10**	S	S	S	S	S	S	S	S	S	R
Ceftriaxone	**CTR30**	S	S	R	R	R	S	S	S	S	S
Cefoperazone	**CPZ75**	S	S	S	R	S	S	S	R	S	S
Piperacillin/Tazobactam	**PIT100/10**	S	S	S	S	S	S	S	S	S	S
Mezlocillin	**MZ75**	R	S	S	S	S	S	S	R	S	S
Aztreonam	**AT30**	S	R	S	S	S	R	S	S	S	S
Netillin	**NET30**	S	S	R	S	S	S	S	S	R	S
Carbenicillin	**CB100**	S	S	S	S	S	R	S	S	S	S
Meropenem	**MRP10**	S	S	S	S	S	S	S	S	R	S
Ceftazidime	**CAZ30**	S	R	S	R	S	S	S	S	S	S
Ceftizoxime	**CZX30**	R	R	R	S	S	S	S	S	R	S
Gatifloxacin	**GAT5**	S	S	S	S	S	S	S	R	S	S
Gentamicin	**GEN10**	S	S	S	S	S	S	S	S	S	S
Tobramycin	**TOB10**	S	S	S	S	S	S	S	S	S	S
	Total	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**
	Sensitive	**14**	**12**	**13**	**13**	**14**	**14**	**15**	**13**	**13**	**14**
	Resistance	**2**	**4**	**3**	**3**	**2**	**2**	**1**	**3**	**3**	**2**
***Note: S- sensitive; R- resistant; NI- No inhibition**											

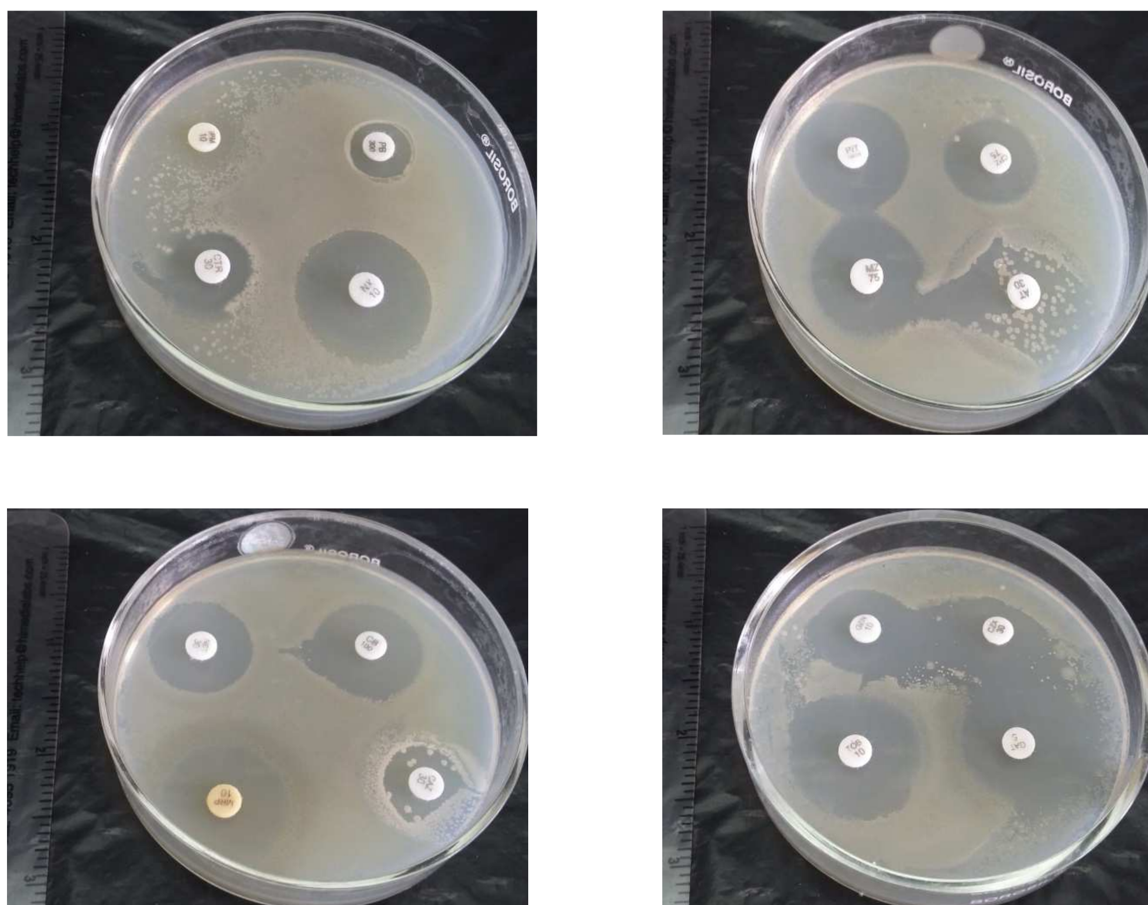


Fig. 3: Antibiotic sensitivity against *P. aeruginosa*

Table 2: Antibiotic sensitivity against *S. enterica*

Antibiotics	**abbreviations**	**Zone of Inhibition (mm)**									
		S. enterica									
Strains		***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Polymixin-B	**PB300**	S	R	S	R	R	S	S	R	R	R
Imipenem	**IPM10**	S	S	S	S	S	S	S	S	S	R
Norfloxacin	**NX10**	S	R	S	R	S	S	S	R	S	S
Ceftriaxone	**CTR30**	S	S	S	R	S	S	S	S	S	R
Cefoperazone	**CPZ75**	R	R	S	S	S	R	S	R	S	S
Piperacillin/Tazobactam	**PIT100/10**	S	S	R	S	S	S	R	S	S	S
Mezlocillin	**MZ75**	S	R	S	S	S	S	S	R	S	S
Aztreonam	**AT30**	S	S	S	S	S	S	R	S	S	R
Netillin	**NET30**	S	S	S	S	S	S	S	R	R	S
Carbenicillin	**CB100**	S	S	R	R	S	S	R	S	R	S
Meropenem	**MRP10**	S	S	S	S	S	R	S	S	R	S

Ceftazidime	**CAZ30**	S	S	S	S	S	R	S	S	R	R
Ceftizoxime	**CZX30**	S	S	S	S	R	S	S	S	S	R
Gatifloxacin	**GAT5**	S	S	S	S	S	S	S	S	S	S
Gentamicin	**GEN10**	S	R	R	S	R	S	R	S	S	S
Tobramycin	**TOB10**	R	S	S	R	S	R	R	S	S	S
	Total	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**
	Sensitive	**14**	**11**	**13**	**11**	**13**	**12**	**11**	**11**	**11**	**10**
	Resistance	**2**	**5**	**3**	**5**	**3**	**4**	**5**	**5**	**5**	**6**
***Note: S- sensitive; R- resistant; NI- No inhibition**											

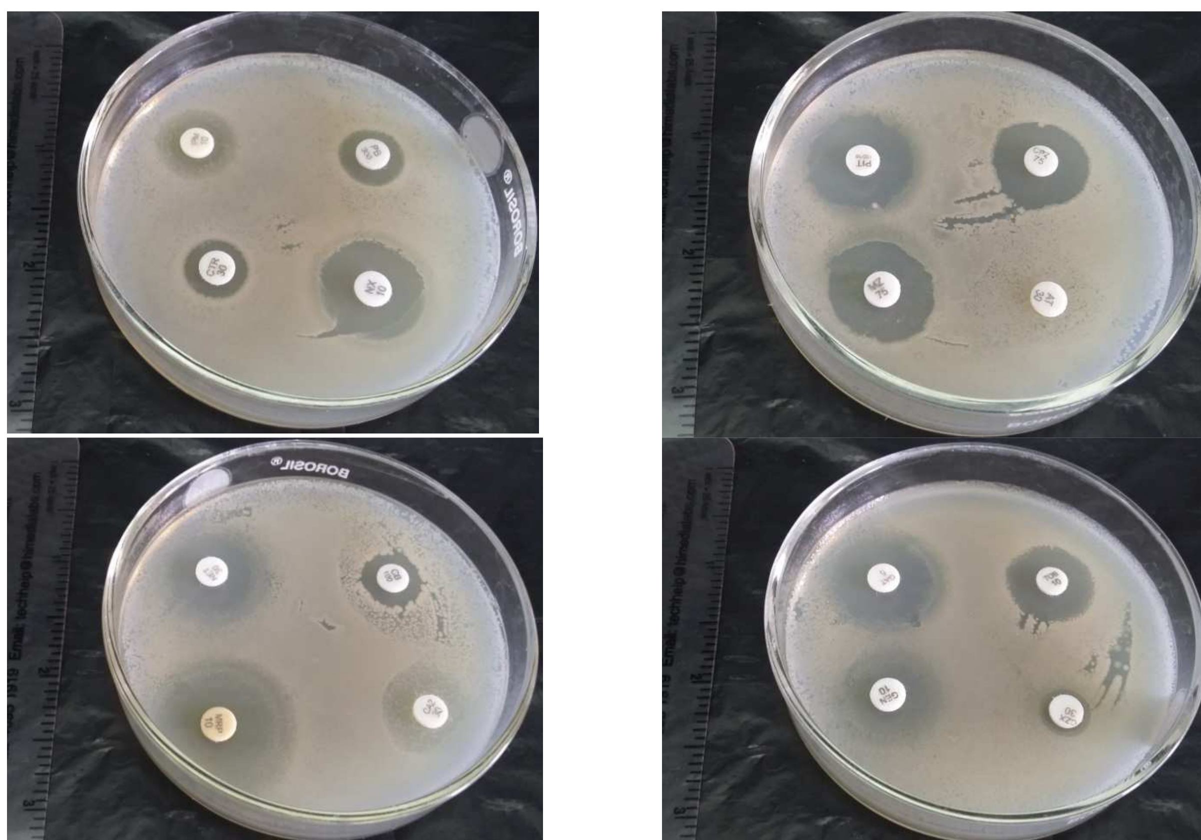


Fig. 4: Antibiotic sensitivity against *S. enterica*

Table 3: Antibiotic sensitivity against *K. pneumoniae*

Antibiotics	**abbreviations**	**Zone of Inhibition (mm)**									
		K. pneumoniae									
Strains		***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Polymixin-B	**PB300**	S	R	S	R	R	S	R	S	S	S
Imipenem	**IPM10**	S	R	S	S	R	S	S	S	S	S
Norfloxacin	**NX10**	R	R	S	R	S	R	S	S	S	S
Ceftriaxone	**CTR30**	S	S	S	S	R	S	S	S	S	R
Cefoperazone	**CPZ75**	R	S	R	S	S	R	S	S	S	S
Piperacillin/Tazobactam	**PIT100/10**	S	S	S	S	S	S	S	R	S	S
Mezlocillin	**MZ75**	S	S	S	S	S	R	R	S	S	S
Aztreonam	**AT30**	S	S	R	S	R	S	R	S	R	S
Netillin	**NET30**	R	S	R	S	S	S	S	S	S	S
Carbenicillin	**CB100**	S	S	S	S	R	S	S	R	R	S
Meropenem	**MRP10**	S	R	S	R	S	R	S	R	S	S
Ceftazidime	**CAZ30**	R	S	S	S	R	R	R	S	S	S
Ceftizoxime	**CZX30**	S	R	R	R	R	S	R	R	R	S
Gatifloxacin	**GAT5**	S	S	R	S	S	S	R	S	S	S
Gentamicin	**GEN10**	S	R	R	S	S	S	S	S	S	S
Tobramycin	**TOB10**	R	S	S	S	S	S	S	S	S	S
	Total	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**
	Sensitive	**11**	**10**	**10**	**12**	**9**	**11**	**10**	**12**	**13**	**15**
	Resistance	**5**	**6**	**6**	**4**	**7**	**5**	**6**	**4**	**3**	**1**
***Note: S- sensitive; R- resistant; NI- No inhibition**											

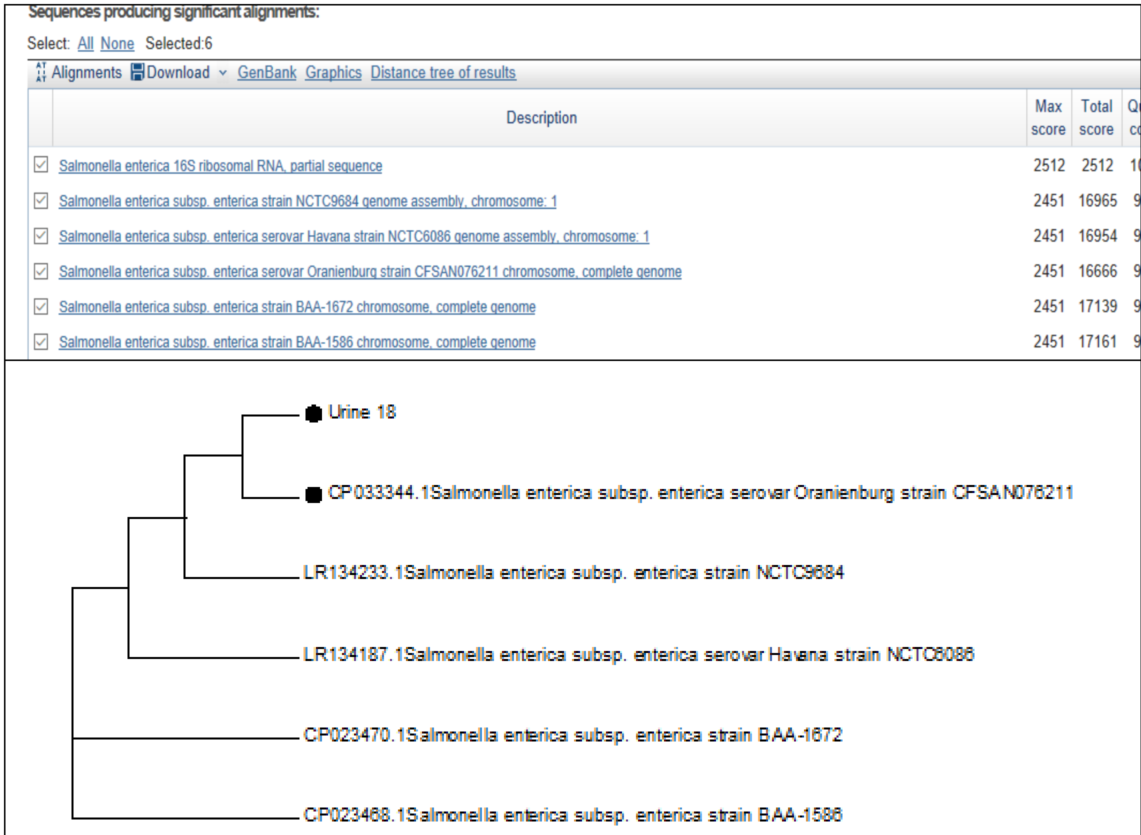
Table 4 : Antibacterial activity of Bel, Hirda and Adulsa against MDR pathogens

Sr. No.	Organism	Zone of Inhibition																															
		BEL												**BEHADA**												**ADULSA**							
		BARK				**LEAF**				**FRUIT**				**BARK**				**LEAF**				**FRUIT**				**BARK**				**LEAF**			
		BBP	BBE	BBM	BBW	BLP	BLE	BLM	BLW	BFP	BFE	BFM	BFW	BeBP	BeBE	BeBM	BeBW	BeLP	BeLE	BeLM	BeLW	BeFP	BeFE	BeFM	BeFW	ABP	ABE	ABM	ABW	ALP	ALE	ALM	ALW
1	***P. aeruginosa 8***	13	13	13	14	NI	15	15	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20	21	NI	NI	15	14	16	10	10	11	10	NI	NI	NI	NI
2	***K. pneumoniae 6***	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	13	12	14	NI	NI	NI	NI	NI	NI	NI	NI
3	***S. enterica 10***	NI	15	NI	17	NI	NI	NI	NI	NI	NI	NI	NI	NI	20	23	19	NI	NI	NI	NI	NI	32	31	30	NI	NI	NI	NI	NI	12	NI	NI

BBP: Bel bark petroleum ether; BBE: Bel bark ethanol; BBM: Bel bark methanol; BBW: Bel bark water; BLP: Bel leaf petroleum ether; BLE: Bel leaf ethanol; BLM: Bel leaf methanol; BLW: Bel leaf water; BFP: Bel fruit Petroleum ether; BFE: Bel fruit ethanol; BFM: Bel fruit methanol;BFW: Bel fruit water; BeBP: Behada bark petroleum ether; BeBE: Behada bark ethanol; BeBM: Behada bark methanol; BeBW: Behada bark water; BeLP: Behada leaf petroleum ether; BeLE: Behada leaf ethanol; BeLM: Behada leaf methanol; BeLW: Behada leaf water;BeFP: Behada fruit petroleum ether; BeFE: Behada fruit ethanol; BeFM: Behada fruit methanol; BeFW: Behada fruit water; ABP: Adulsa bark petroleum ether; ABE: Adulsa bark ethanol; ABM: Adulsa bark methanol; ABW: Adulsa bark water; ALP: Adulsa leaf petroleum ether; ALE: Adulsa leaf ethanol; ALM: Adulsa leaf methanol; ALW: Adulsa leaf water; NI: No Inhibition.

Table 5: Antibiotic sensitivity assay against given plant extracts (synergistic)

Sr.No.	Extracts	Organisms	Antibiotics							
			IPM	AT	CAZ	CZX	PB	CTR	MZ	CB
1	BBP	*P. aeruginosa*	13	23	10	19				
2	BBP	*Salmonella enterica*	13	27	22	30	13	27		
3	BBE	*P. aeruginosa*	13	27	19	10				
4	BBE	*Salmonella enterica*	NI	NI	NI	18	17	20		
5	BBM	*P. aeruginosa*	16	29	NI	16				
6	BBM	*Salmonella enterica*	15	14	19	13	15	28		
7	BBW	*P. aeruginosa*	15	24	NI	13				
	BBW	*Salmonella enterica*	19	NI	18	28	15	23		
8	BLE	*P. aeruginosa*	20	21	14	11				
9	BLM	*P. aeruginosa*	12	NI	12	12				
10	BeBE	*Salmonella enterica*	19	27	NI	26	12	20		
11	BeBM	*Salmonella enterica*	24	NI	18	13	12	20		
12	BeBW	*Salmonella enterica*	16	36	17	35	11	18		
13	BeLE	*P. aeruginosa*	NI	NI	14	23				
14	BeLM	*P. aeruginosa*	NI	29	30	31				
15	BeFE	*Salmonella enterica*	NI	28	21	29	11	27		
16	BeFE	*P. aeruginosa*	11	NI	NI	14				
17	*BeFE	*K. pneumoniae*	14	16	25	29	12	27		32
18	BeFM	*P. aeruginosa*	NI	NI	10	10				
19	BeFM	*K. pneumoniae*	NI	NI	NI	17	12	15		NI
20	BeFM	*Salmonella enterica*	NI	10	10	11	12	27		
21	BeFW	*P. aeruginosa*	19	20	NI	25				
22	BeFW	*K. pneumoniae*	17	11	NI	12	12	19		NI
23	BeFW	*Salmonella enterica*	16	NI	NI	13	12	20		
24	ABP	*P. aeruginosa*	16	28	12	16				
25	ABE	*P. aeruginosa*	12	NI	12	18				
26	ABM	*P. aeruginosa*	13	27	20	16				
27	ABW	*P. aeruginosa*	23	22	20	15				
28	ALE	*Salmonella enterica*	16	NI	18	30,12	15	23		
29	ALW	*Salmonella enterica*	19	15	13	27	14	22		



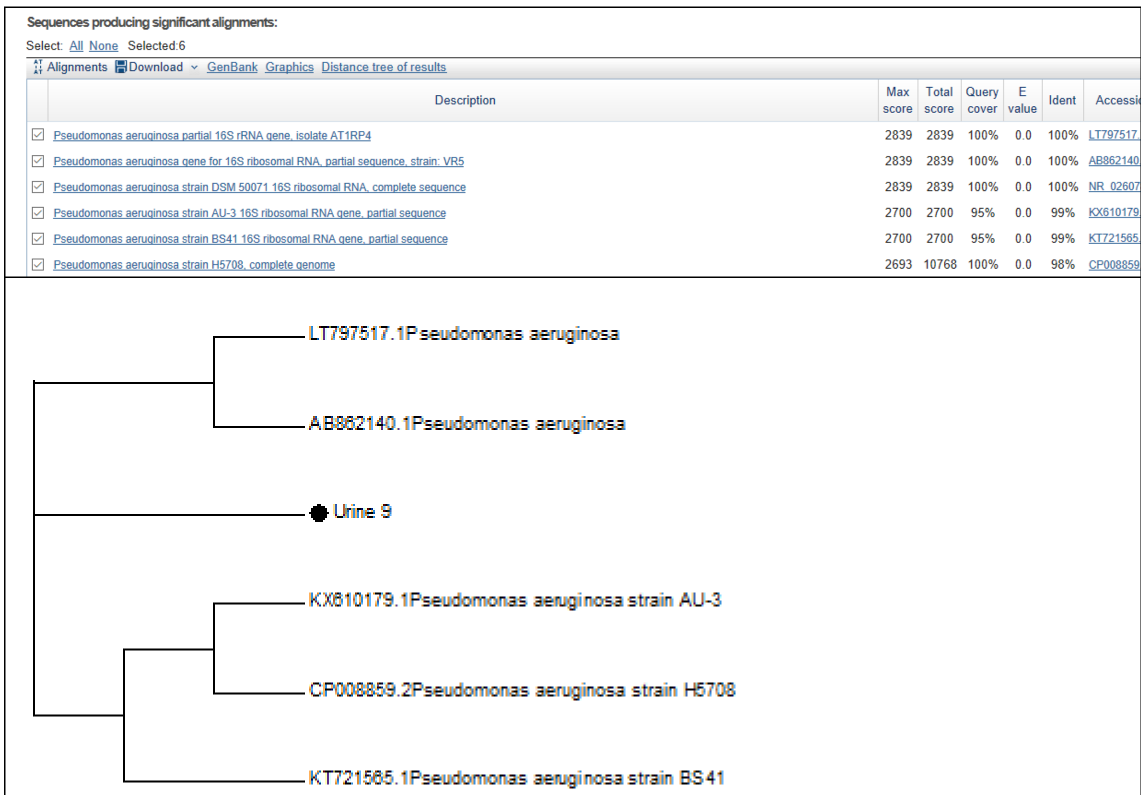
Sequences producing significant alignments:

Select: All None Selected:6

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Q co
Salmonella enterica 16S ribosomal RNA, partial sequence	2512	2512	1
Salmonella enterica subsp. enterica strain NCTC9684 genome assembly, chromosome: 1	2451	16965	9
Salmonella enterica subsp. enterica serovar Havana strain NCTC6086 genome assembly, chromosome: 1	2451	16954	9
Salmonella enterica subsp. enterica serovar Oranienburg strain CFSAN076211 chromosome, complete genome	2451	16666	9
Salmonella enterica subsp. enterica strain BAA-1672 chromosome, complete genome	2451	17139	9
Salmonella enterica subsp. enterica strain BAA-1586 chromosome, complete genome	2451	17161	9

Fig. 6 : Urine 18 sample idenitfied as *Salmonella enterica* confirmed by BLASTN and Phylogenetic analysis.



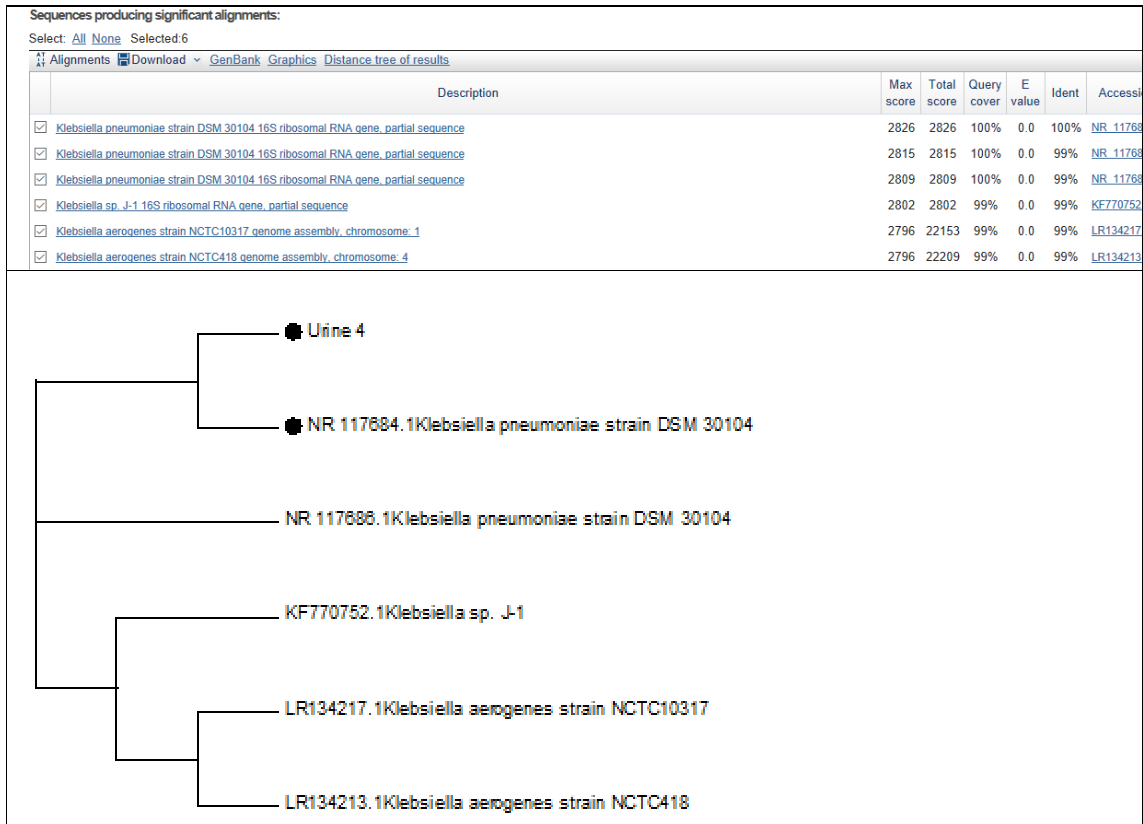
Sequences producing significant alignments:

Select: All None Selected:6

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accessi
Pseudomonas aeruginosa partial 16S rRNA gene, isolate AT1RP4	2839	2839	100%	0.0	100%	LT797517.
Pseudomonas aeruginosa gene for 16S ribosomal RNA, partial sequence, strain: VR5	2839	2839	100%	0.0	100%	AB862140
Pseudomonas aeruginosa strain DSM 50071 16S ribosomal RNA, complete sequence	2839	2839	100%	0.0	100%	NR_02607
Pseudomonas aeruginosa strain AU-3 16S ribosomal RNA gene, partial sequence	2700	2700	95%	0.0	99%	KX610179
Pseudomonas aeruginosa strain BS41 16S ribosomal RNA gene, partial sequence	2700	2700	95%	0.0	99%	KT721565
Pseudomonas aeruginosa strain H5708, complete genome	2693	10768	100%	0.0	98%	CP008859

Fig. 7: Urine 9 sample idenitfied as *Pseudomonas aeruginosa* confirmed by BLASTN and Phylogenetic analysis.



Sequences producing significant alignments:

Select: All None Selected:6

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accessi
Klebsiella pneumoniae strain DSM 30104 16S ribosomal RNA gene, partial sequence	2826	2826	100%	0.0	100%	NR_11768
Klebsiella pneumoniae strain DSM 30104 16S ribosomal RNA gene, partial sequence	2815	2815	100%	0.0	99%	NR_11768
Klebsiella pneumoniae strain DSM 30104 16S ribosomal RNA gene, partial sequence	2809	2809	100%	0.0	99%	NR_11768
Klebsiella sp. J-1 16S ribosomal RNA gene, partial sequence	2802	2802	99%	0.0	99%	KF770752
Klebsiella aerogenes strain NCTC10317 genome assembly, chromosome: 1	2796	22153	99%	0.0	99%	LR134217
Klebsiella aerogenes strain NCTC418 genome assembly, chromosome: 4	2796	22209	99%	0.0	99%	LR134213

Fig. 8: Urine 4 sample idenitfied as *K. pneumoniae* confirmed by BLASTN and Phylogenetic analysis.