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Susceptibility Pattern of *Pseudomonas aeruginosa* Against Antimicrobial Agents and Some Plant Extracts with Focus on its Prevalence in Different Sources

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Abstract: The present study was performed to isolate, characterize and evaluate the prevalence of *Pseudomonas. aeruginosa* in 340 samples of different sources (human, chicken, animals and environment), as well as detection of haemolysin production and determing its susceptibility to different antimicrobials. The antimicrobial activity of some plant ethanol extract on *P. aeruginosa* was also studied. 69 samples out of 340 were found to be positive to *P. aeruginosa* with a pecentage of 20.3%, 65strains out of these positive isolates were only gave positive haemolysin activity. The *in vitro* antimicrobial sensetivity results revealed that all the 69 isolated strains showed high resistance to amoxycillin and tobramycin, only 2 strains exhibited moderate sensitivity to cephalexin (2.9). However, these isolated strains varied in their sensitivity to Amikacin (40.6%), Impenem (18.8%), Meropenem (20.3%), gentamicin (1.4%), ciprofloxacin (17.4%), ofloxacin (52.2%), trimethoprim - sulfamethazol (2.9%) and cefoperazone (7.2%). Results of the tested plant extracts showed that Clove (*Syzygium aromaticum*) ethanol extract at concentration 100 mg/ml exerts growth inhibitory activity on 50 *P.*aeruginosa isolated strains with inhibitory zone ranged from 12.4-16.4 mm. Eucalyptus (*Eucalyptus globulus*) extract at concentration 100 mg/ml showed high antibacterial activity against 61 isolated strains (inhibition zone 15.09-18.35 mm), meanwhile at concentration 40 mg/ml showed moderate activity against only 24 strains (inhibition zone 9.4-10.2). Hibiscus (*Hibiscus sabdariffa*) had lowered activity against only 7 strains with growth ihibitory zone 9.5-10.6 mm. It could be concluded that the incidence of antimicrobial sensitivity for *P. aeruginosa* was varied for each individual antimicrobial depending on the isolate strain and source of isolation. Each of Clove and Eucalyptus ethanol extracts (100 mg/ml) have great potential antibacterial activity against *P. aeruginosa*, however Hibiscus ethanol extract exhibits more lesser activity than them.

Key words: Plant extracts · Antimicrobials · Haemolysin · Bacterial resistance

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic organism which is prevalent in water, it is a classic opportunistic pathogen as it initiates resistance to many antibiotics and disinfectant, in addition to its armory of putative virulence factors plus plasmid acquired resistance [1].

It is the most common Gram-negative bacterium found in nosocomial infections causing various spectra of infections, especially in neutropenic, immunocompromised, burns/tissue injury and cystic fibrosis patients all over the world [2].

In animals, *P. aeruginosa* has been assigned as the distinct cause of otitis externa [3], cystitis, endocarditis, dermatitis, wound infections, conjunctivitis, equine metritis and ulcerative keratoconjunctivitis, mink haemorrhagic pneumonia, deep pyoderma, infections of the lower urinary tract [4], prostatitis, osteomyelitis, chronic rhinitis, pleuritis, mastitis [5] septicaemia [6]. This organism had also been identified as animal pathogen and as the occasional cause of bovine mastitis [7], *P.aeruginosa* also is most often associated with sporadic clinical mastitis among sheep and goat [8].

Since the majority of *P. aeruginosa* strains are resistant to most of antibacterial agents, it is

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considered as one of the major problems in many hospitals [9]. Its high rates for developing resistance against most of the antimicrobials agents initiate a great need for finding other alternative medicine towered it [10, 11]. Many plants are reported to possess antibacterial, antifungal and immunosuppressive activities which would be useful to clinical uses [12, 13]. However, these are not investigated against *P. aeruginosa*.

The current study was therefore designed to shed some light on:

- Isolation and identification of *P. aeruginosa* isolated from human, animal and environmental sources.
- Investigation of one of virulence factors associated with *P. aeruginosa* (hemolysis on Blood agar).
- Performing Antibiogram studies using different type of antibacterial agents.
- Studying the antibacterial activity of some medicinal plant extracts on *P. aeruginosa*.

MATERIALS AND METHODS

Samples: A total of 340 samples of which 150 human (50 sputum, 50 urine and 50 wound swab). 100 poultry swab samples (20 nasal cavity, 15 nasal sinus, 16 lung, 39 endotracheal and 10 wound swabs). 10 apparent healthy goats (milk), 5 apparent healthy cattle (urine). 15 dog (5 apparent healthy, urine; 10 diseased, wound swab). 19 water samples (11 tap water and 9 water for renal dialysis) and finally 40 air samples from rooms of hospitals and intensive care units was collected. All previous swabs and samples were inoculated into Nutrient broth as transported media to keep the viability of organism and incubated aerobically at 37°C for 24 - 48 hours.

Bacteriological Examination

Isolation of *P. Aeruginosa* from Different Collected Samples: A loopfull from each inoculated nutrient broth was transferred and cultivated onto the surface of pseudosel, blood and nutrient agars. All of the inoculated plates were incubated at 37°C for 24-48 hours.

Identification of the Isolates: The suspected colonies were examined for their colonial morphology, haemolytic activity, microscopical examination and biochemical character [14, 15].

Antibacterial Sensitivity Testing (Disc Diffusion Test): Antibacterial sensitivity test was performed using subcultures of *P. aeruginosa* isolates [16].

Determination of Multiple Antibiotic Resistance Index (MAR Index): The multiple antibiotic resistance index MAR index was determined for each isolate by dividing the number of antibiotics against which the isolate showed resistant over the total number of antibiotics tested [17].

$$\text{MAR index} = \frac{\text{Number of antibiotics against which isolate showed resistance}}{\text{Total number of antibiotics tested}}$$

MAR index higher than 0.3 indicates wide use of this antibiotic in the originating environment of this isolate [18].

Preparation of Plant Extracts: 150 grams of air dried powdered of each tested plants were extracted separately till exhaustion by percolation several times with ethanol (95%) as a solvent. The solvent was removed by rotatory evaporator apparatus at 70°C [19], the obtained extracts then left at 37°C for 48 hrs for complete dryness of each extract. Each extract was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent) for yielding concentrations of 100, 40, 20 and 10 mg/ml from each extract.

Determination of the Antibacterial Activity of Plant Extracts: The antibacterial activity of the tested plant extracts were studies in vitro using the hole plate diffusion method [20].

RESULTS

Incidence of *P. aeruginosa* among the Examined Samples: A total of 340 samples from human, animal, poultry, environment was examined for detection of *P. aeruginosa*. The result showed that 69 samples were positive for *P. aeruginosa*, with a percentage of 20.3% (Table 1).

Indentification of *P. aeruginosa* Isolates: Results of identification of 69 *P. aeruginosa* Strains showed that 65 strains gave positive hemolysin activity and cause hemolysis on blood agar. It showed beta -hemolytic colonies on blood agar with incidence of 94.2%, the recorded four negative isolates were classified as 3 water isolates and 1 human skin isolate.

Table 1: The relationship between *P. aeruginosa* recovery rate and the source of isolation

Origin	Source of isolation	Number of samples	Number of isolates	Percentage
Human	Respiratory tract	50	12	24.
	Urinary tract	50	5	10.
	Wound	50	8	16.
Poultry	Respiratory tract	90	28	31.
	Skin	10	2	20.
Animal	Cattle's urine	5	1	20.
	Goat's milk	10	2	20.
	Dog's urine	10	1	10.
	Dog's skin	5	1	20.
Environment	Air	40	3	7.5
	Tap water	11	4	36.364
	Water of renal dialysis	9	2	22.22.
Total		340	69	20.3

Table 2: Results of *In vitro* Antibiogram sensitivity test using disc diffusion technique on 69 strains of *P. aeruginosa*

	Resistance		Moderate		Sensitive	
Antimicrobial	No	%	No	%	No	%
AK amikacin 30	30	43.5	11	15.9	28	40.6
AMX amoxycilln 10	69	100.0	0	0.0	0	0.0
KZ cephalexin 30	67	97.1	2	2.9	0	0.0
IPM impenem 10	50	72.5	6	8.7	13	18.8
MEM meropenem 10	50	72.5	5	7.2	14	20.3
CN gentamicin 10	65	94.2	3	4.4	1	1.4
CIP ciprofloxacin 10	55	79.7	2	2.9	12	17.4
OFX ofloxacin 10	29	42.0	4	5.8	36	52.2
SXT trimethoprimsulfamethazol 25	53	76.8	14	20.3	2	2.9
CFP cefoperazone 10	63	91.4	1	1.4	5	7.2
TOB tobramycin 10	69	100.0	0	0.0	0	0.0

***In vitro* Antibiogram Sensitivity Study Using Disc Diffusion Technique on 69 Strains of *P. aeruginosa*:** The *in vitro* sensitivity of 69 *P. aeruginosa* isolates was carried against 11 chemotheraputic agents. Results showed that all of the 69 isolates exhibited complete resistant to amoxicillin and tobramycin. Only 2 strain showed moderate sensitivity to cephalexin with a sensitivity index of 2.9%. The rest 67 strains were completely sensitive to the remaining of the selected antibacterials (amikacin 40.6%, impenem 18.8%, meropenem 20.3%, gentamicin 1.4%, ciprofloxacin 17.4%, ofloxacin 52.2%, trimethoprim-sulfamethazol 2.9% and cefoperazone 7.2%) (Table 2).

Human isolates showed complete resistant to Tobramycin, cefoperazone, amoxicillin, cephalexin, gentamicin and trimethoprim-sulfamethazol. While these were completly sensitive to amikacin 68%, impenem 52%, meropenem 52%, ciprofloxacin36% and ofloxacin 32% (Figure 1).

Poultry isolates exhibited complete resistant to tobramycin, amoxicillin, ciprofloxacin and meropenem. While these were sensitivite to Amikacin 23.33%, gentamicin 3.33%, ofloxacin 73.34%, trimethoprim-sulfamethazol 3.33% and cefoperazone 13.33%, cephalexin and impenem 3.33% (Fig. 2).

Animal isolates were completly resistant to tobramycin, impenem, meropenem, gentamicin, ciprofloxacin, amoxicillin, cephalexin and trimethoprim-sulfamethazol.However these were sensitive to amikacin 20%, ofloxacin 80% and cefoperazone 20% (Fig. 3).

Environmental isolates were completely resistant to tobramycin, cefoperazone, amoxicillin, gentamicin and cephalexin. While were completely sensitive to amikacin, meropenem,ciprofloxacin, ofloxacin and trimethoprim-sulfamethazol with incidence of 33.4, 11.1, 33.3, 22.25 and 11.1 %, respectively. However, moderate sensitivity was recorded to impenem with incidence percent of 11.1% (Fig. 4).

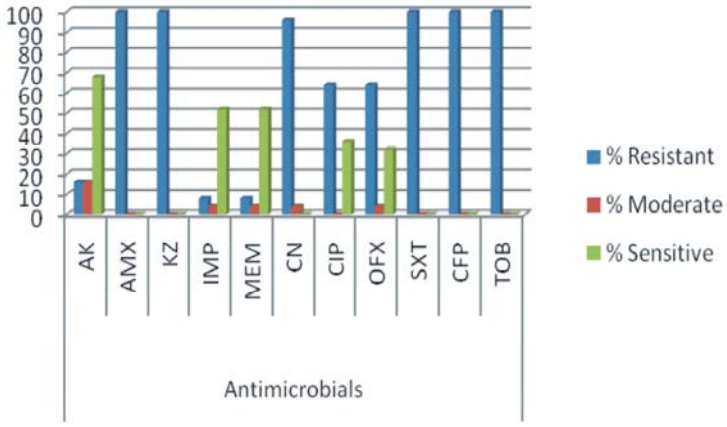

Fig. 1: Antibiogram study on 25 isolates of *P.aeruginosa* human strains

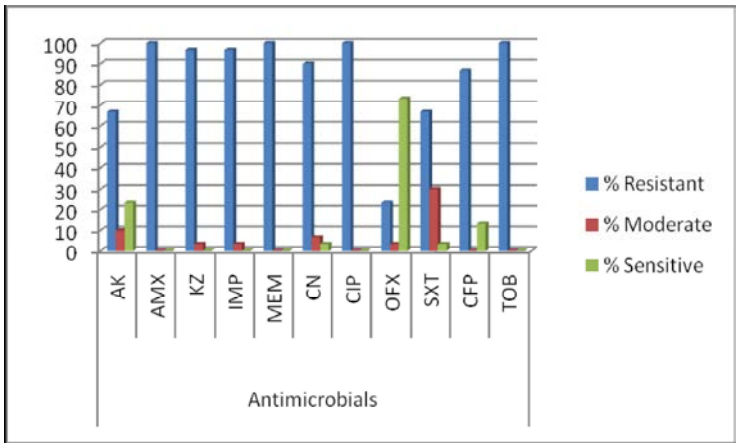

Fig. 2 : Antibiogram study on 30 isolates of *P.aeruginosa* poultry strains

AK: Amikacin 30 AMX: Amoxicillin10 KZ: Keflex (cephalexine)30
IMP: Impenem 10 MEM: Meropenem10 CN: Gentamicin 10
CIP: Ciprofloxacin10 OFX: Ofloxacin10 TOB: Tobramycin 10
SXT: Trimethoprim-sulfamethazol 25 CFP: Cefoperazone10
* The concentrations of the used antimicrobial discs are represented as µg.

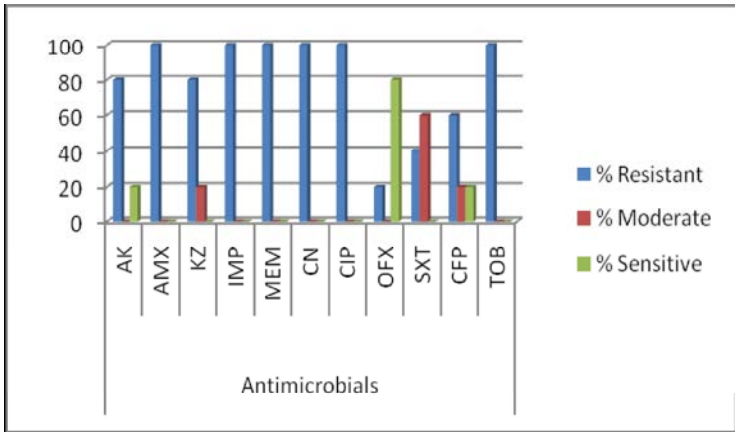

Fig. 3: Antibiogram study on 5 isolates of *P.aeruginosa* animal strains

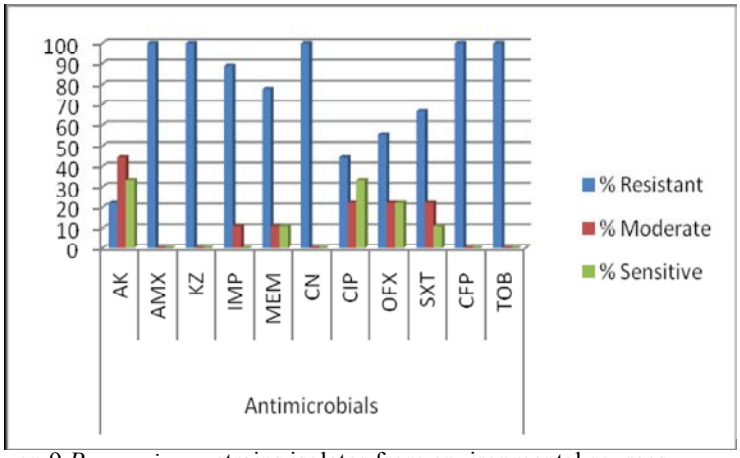

Fig. 4: Antibiogram study on 9 *P.aeruginosa* strains isolates from environmental sources

AK: Amikacin 30 AMX: Amoxicillin10 KZ: Keflex (cephalexine) 30
IMP: impenem 10 MEM: Meropenem10 CN: Gentamicin 10
CIP:ciprofloxacin 10 OFX: Ofloxacin10 TOB: Tobramycin 10
SXT: trimethoprim-sulfamethazol 25 CFP: Cefoperazone10
* The concentrations of the used antimicrobial discs are represented as µg

Table 3: Mean values of the recorded inhibitory zones of Clove (*Syzygium aramaticum*) ethanol extract against 69 *P. aeruginosa* strains at different tested concentrations

		Zones of inhibition (mm) .			
Kind of sample	No of isolates	100mg/mlml	40mg/ml	20mg/ml	10mg/ml
Poultry	11	12.8	-	-	-
Poultry	15	14.3	-	-	-
Poultry	2	15.2	-	-	-
Dog	1	16.4	-	-	-
Human	7	16.3	-	-	-
Human	7	15.7	-	-	-
Human	3	12.4	-	-	-
Water	4	14.6	-	-	-
Poultry	2	0	-	-	-
Goat	2	0	-	-	-
Dog	1	0	-	-	-
Cattle	1	0	-	-	-
Human	8	0	-	-	-
Water	2	0	-	-	-
Air	3	0	-	-	-

Antibacterial Activity of Plant Extracts Against *P. aeruginosa* Isolated Strains: *In vitro* antibacterial sensitivity test was performed for the 69 *P. aeruginosa* isolated strains against 8 ethanol plant extracts. The result showed that Harmel (*Peganum harmala*), Rosemary (*Rosmarinus Officinalis*), Tumeric (*Curcumal longa*), Marjoram (*Origanum vulgare*), Thyme (*Thymus vulgaris*), Ginger (*Zingiber officinale*) extracts have no any antibacterial activity at any tested concentration against these 69 isolated strains. While, ethanol extracts of Clove (*Syzygium aromaticum*), Eucalyptus (*Eucalyptus globules*) and Hibiscus (*Hibiscus sabdariffa*) plants exhibited antibacterial activity against these isolated tested strains.

Clove (Syzygium aromaticum) ethanol extract in conc. Of 100mg/ml exerts antibacterial activity against 50 strains among the isolated *P. aeruginosa* strains (8 strains with inhibitory zone 16.3-16.4 mm, 9 strains with inhibitory zone 15.2-15.7 mm, 19 strains with zone 14.3-14.6 mm and 14 strains with zone 12.2 - 12.8 mm). However, the lowered concentrations (40, 20 and 10 mg/ml) showed no any antibacterial activity against all the tested isolates (Table 3).

Table 4: Mean values of the recorded inhibitory zones of Eucalyptus (*Eucalyptus globules*) ethanol extract against 69 *P. aeruginosa* strains at different tested concentrations

		Zones of inhibition (mm).			
Kind of sample	No of isolates	100mg/ml	40mg/ml	20mg/ml	10mg/ml
Poultry	7	18.35	-	-	-
Poultry	8	15.62	10.12	-	-
Poultry	5	15.33	-	-	-
Poultry	2	17.51	9.35	-	-
Poultry	8	15.82	-	-	-
Goat	2	16.22	9.36	-	-
Dog	1	15.35	-	-	-
Cattle	1	16.28	-	-	-
Human	12	15.53	-	-	-
Human	3	18.12	-	-	-
Human	4	17.38	10.2	-	-
Human	6	17.22	9.4	-	-
Water	2	15.09	9.5	-	-
Dog	1	-	-	-	-
Water	4	-	-	-	-
Air	3	-	-	-	-

Table 5: Mean values of the recorded inhibitory zones of Hibiscus (*Hibiscus sabdariffa*) extract against 69 *P. aeruginosa* strains at different tested concentrations

		Zones of inhibition (mm)			
Kind of sample	No of isolates	100mg/ml	40mg/ml	20mg/ml	10mg/ml
Poultry	3	-	-	-	-
Poultry	5	10.8	-	-	-
Poultry	6	-	-	-	-
Poultry	8	-	-	-	-
Dog	2	9.4	-	-	-
Cattle	1	-	-	-	-
Human	7	-	-	-	-
Human	5	-	-	-	-
Human	6	-	-	-	-
Poultry	8	-	-	-	-
Human	7	-	-	-	-
Goat	2	-	-	-	-
Water	6	-	-	-	-
Air	3	-	-	-	-

Eucalyptus (*Eucalyptus globules*) ethanol extract at conc. of 100mg/ml gave high antibacterial activity against 61 *P. aeruginosa* strains (10 strains with inhibitory zone 18.12-18.35 mm, 12 strains with zone 17.22-17.5mm, 3 strains with zone 16.22-16.28mm and 36 strains with zone 15.09-15.82 mm). Meanwhile, in a concentration of 40 mg/ml it showed intermediate activity against 24 strains (12 strains with zone 10.12-10.2 mm and 12 strains with zone 9.35-9.4 mm). However, at concentrations of 20 and 10 mg/ml eucalyptus extract showed no any antibacterial activity against any of the 69 isolated strains (Table 4).

Hibiscus (*Hibiscus sabdariffa*) ethanol extract at conc. 100mg/ml demonstrated lowered antibacterial activity only against 7 isolates (5 strains with zone 10.8 mm and 2 strains with zone 9.4mm). The lowered concentrations showed no any antibacterial activity against the tested strains (Table 5).

DISCUSSION

In this study a total number of 340 samples, classified as 30 animal samples, 150 human,100 poultry and 60 environmental samples were subjected to bacteriological examination for detection and isolation of *P. aeruginosa* strains. The incidence of *P. aeruginosa* among the examined samples were 69 positive isolates with a percentage of 20.3%. According to the source of samples the incidence of isolation were 16.67, 30, 16.67 and 15% for the human, poultry, animal and environmental samples, respectively.

Regarding to human isolates, the recovery rate from respiratory tract in our results (24%) was higher than that recorded in pneumonia suffering patients (18.7% and 13%) determined by Mathai *et al*. [21] and Mastoraki *et al*. [11] respectively. In the present study the incidence of *P. aeruginosa* among the examined human urine samples was 5 out of 50 examined ones with a percentage of 10%. This was low than 41.3% that was recorded through a retrospective one year analysis for urine samples of intensive care unite patients [22].

Our results recorded 8 positive *P. aeruginosa* isolates from a total 50 wound swab samples with an incidence of 16% which is similar to that obtained by Koffi *et a*l. [23] in 12 patients suffered from poses abscess.

Regarding to poultry isolates in our study *P. aeruginosa* was isolated from poultry respiratory tract with a percentage of 31%. Lower incidence of isolation 7.4% and 11.5% were recorded by Riad [24] and Abd-Allah [25], respectively.

In the present studuy, *P. aeruginosa* was isolated with incidence of 20% from both cattles urine and goats milk samples. Lower prevalence (0.5 - 1.6%) were recorded for both urine samples of urinary tract infected calves [26] and milk samples (12.4%) from udders of mastitis suffering dairy goats [27].

Results of isolation from environmental samples (water and air) revealed that only 9 samples gave positive isolates from a total 60 samples with incidence of 15%. These samples were classified as tap water (36.364%), water bottles used for renal dialysis (22.22%). Regarding the air samples, only 3 ones were positive for *P. aeruginosa* from a total 40 examined samples with incidence of 7.5%. Barben *et al*. [28] previously isolated *P. aeruginosa* from water specimens of 46 pools with incidence of 7%.

P. aeruginosa produces a number of exoenzymes, which have been implicated as virulence factors in infections caused by this organism, hemolysin is one of these exoenzymes [29]. Previously hemolysin is considered as important virulence factor of *P. aeruginosa* [30], but it has been established that hemolysin production is dependent on the antigenic structure of *P. aeruginosa* and the type of isolate specimens [29]. As the virulence of *P. aeruginosa* is multifactorial and has been attributed to cell-associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin-A, exoenzyme-S, hemolysins (rhamnolipids) and siderophores [31, 32]. In our study 65 out of 69 *P. aeruginosa* strains gave positive hemolysin activity and caused hemolysis on blood agar, beta hemolytic colonies were developed with incidence of 94.2%. The recorded four negative isolates were isolated from 3 water samples and one human skin specimen. Our results revealed that there are no any significant differences in hemolysin activity among the isolated strains from different sample types, which confirm the previous suggestion of Eliana *et al*. [33] that hemolysin production must not always coincide as a virulence marker for pathogenesity between different *P. aeruginosa* strains.

It is well known that over use of antibiotics has become the major factor for the emergence and dissemination of multi-antibiotic resistant strains of several micro-organisms, in these regards *P.aeruginosa* antibiotic resistance was arised from both intrinsic and acquired resistance. Intrinsic resistance resulted from multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes (e.g. *mexAB-oprM*, *mexXY* etc.) and the low permeability of the bacterial cellular envelopes [34]. Acquired resistance developed either by mutation in chromosomally-encoded antibiotic resistance genes, or by the horizontal gene transfer of antibiotic resistance determinants [35]. Some recent studies [36] has shown that phenotypic resistance associated to biofilm formation or to the emergence of small-colony-variants may be important in the response of *P. aeruginosa* populations to antibiotics treatment.

In vitro sensitivity of 69 *P. aeruginosa* isolates was carried against 11 chemotherapeutic agents. All of the tested isolates showed complete resistant to amoxicillin and tobramycin, only 2 strains have moderate sensitivity (2.9%) to cephalexin. However these strains varied in its sensitivity to Amikacin (40.6%), Impenem (18.8%), Meropenem (20.3%), gentamicin (1.4%), ciprofloxacin (17.4%), ofloxacin (52.2%), trimethoprim - sulfamethazol (2.9%) and cefoperazone (7.2%) as shown in Table 2. Similar results had also been reported for complete resistance of *P. aeruginosa* against amoxicillin (99%) [37].

Longford *et al*. [38] found that 95% of *P. aeruginosa* gentamicin resistant strains were also resistance to polymyxin - B, however these strains were 100% sensitive to amikacin, 85% to streptomycin, 78% to tetracycline and 59% to chloramphenicol.

The data of MYSTIC program show that in Europe, 36.7% of *P. aeruginosa* strains were resistant to ciprofloxacin and 37.2% in USA [39]. Much more ciprofloxacin resistant strains were recorded in this present study (79.9%). Which was higher than 20% and 52.7% previously recorded by Astal[40] and Greta *et al*. [41], respectively. However in various studies conducted in Pakistan [42, 43] more than 90% of *p.aeruginosa* isolates were sensitive to ciprofloxacin. The increased resistance to flouroquinolones is basically a reflection to mutation which is a result of selective pressure created by its over use [44]. There is a great possibility for transmissible genetic elements that encode proteins that block the active site of quinolones [45].

The *In vitro* antibiogram sensitivity test in this study revealed that amikacin, imipenem and meropenem are the most effective antibiotics against human isolates with incidence of 68, 52 and 52%, respectively. These are in accordance with the finding that the most active agents against *P. aeruginosa* were imipenem (84.1%), meropenem (71.6%), amikacin (71.0%) during the first four years of SENTRY program which performed in Latin American Medical Center [10]. The sensitivity of *P. aeruginosa* isolates of urinary tract to amikacin was found to be (91.3 - 93.8%) > tobramycin > meropenem > piperacillin > tazobactam > gentamicin > cefepime (80.0 - 81.8%)as recorded by Mathai *et al.* [21]. Imipenem and meropenem are belonging to carbapenem group of antibacterials which have ability to binds with different penicillin binding proteins, imipenem is the most active drug against *Pseudomonas aeruginosa* found in CSOM [39]. Sensitivity of *P. aeruginosa* to imipenem was studied by Tahira mansoor *et al*. [46] and they revealed that 76% of isolates were found to be sensitive, 17% showed intermediate pattern and only 7% were found to be resistant in the same study. In spite of these established sensitivity of *P. aeruginosa* to imipenem, lowered prevalence resistance (23.9 and 22.9 %) were obtained against it [41, 47]. Prolonged treatment with imipenem in *Pseudomonas aeruginosa* infected patients has often allowed the emergence of resistant mutants against it [48].

Belonging to meropenem, 11.3%of *P. aeruginosa* strains isolated in the study of Tahira Mansoor *et al*. [46] were found to be resistance to meropenem. According to the data of the SENTRY program, 5.1-8.4% of *P. aeruginosa* strains obtained in Canada were resistant to meropenem, 10.2-26.2% in Europe and 7.6-9.0% in USA [10, 49].

Cefoperazone was the most effective one among the third generation cephalosporines against *P. aeruginosa* [37], as only 37% isolates were resistant to it. This finding are consistence with other findings of Gencer *et al.* [50]. In contrary the human isolates in this present study showed 100 % resistance to cefoperazone (cefobid) that might be contribute to production of extended-spectrum β -lactamases (ESBLs) and other enzymes which is previouly showed for ceftazidime resistance [51].

Poultry strains in this study were mainly sensitive to amikacin (23.33%), gentamicin (3.33%), ofloxacin (73.34%), trimethoprim - sulgamethazol (3.33%) and cefoperazone (13.33%). previously *P. aeruginosa* chicken isolates were showed 100% sensitivity to genatmicin and amikacin recorded by Kim *et al*. [52].

Concerning to emergence resistance of *P. aeruginosa* to gentamicin Rezaee *et al*. [53] reported that the mucoid strains of *P. aeruginosa* are more resistant to amikacin, gentamicin and tobramycin than the non-mucoid ones. These mucoid strains have alginate which an extracellular glycocalyx, acts as an ionic barrier against penetration of aminoglycoside antibiotics [54]. Moreover, the alginate-to-anti-biotic ratio could greatly influence the perceived permeability barrier [55]. When this ratio is high, aminoglycosides (but not β-lactams) are retained in the alginate layer. However, low alginate-to-antibiotic ratios quickly result in disruption of the gel structure and faster the penetration of aminoglycosides. Scince there is a suggestion that high levels of antibiotic can saturate the negative charge of align [53].

Water isolates in this study were sensitive to amikacin, oflaxacin, ciprofloxacin, trimethoprim - sulfamethazol and meropenem. High resistance degree was recorded to *P. aeruginosa* isolates obtained from mineralized drinking water toward amoxicillin, trimethoprim and amikacin [56].

The MAR index analysis for each individual *P. aeruginosa* isolated strain in this present study was higher than 0.3, indicating wide antibiotic use in the originating environment of these strains [17, 18].

Results of the *in vitro* sensitivity test of 69 *P. aeruginosa* strains isolated from different animal species, human, water and air were examined against each of the tested ethanol plant extracts revealed that neither harmel *(Peganum harmala)*, rosemary (*Rosmarinus Officinalis)*, tumeric *(Curcumal longa)*, marjoram *(Origanum vulgare)*, thyme *(Thymus vulgaris)* nor ginger *(Zingiber officinale)* had any antibacterial activity, at any tested concentrations, against these isolates. On the conversers each of clove *(Syzygium aromaticum)*, eucalyptus

(Eucalyptus globules) and hibiscus *(Hibiscus sabdariffa)* extracts showed growth inhibitory activity on *P. aeruginosa*.

Clove ethanol extract in concentration of 100mg/ml gave high antibacterial activity against 50 *P. aeruginosa* isolates with growth inhibitory zone 12.8-16.3mm, while the lowered concentrations exhibit no any antibacterial activity. Substintiating to this obtained results clove ethanol extract had been previously showed the highest antimicrobial potentials among several tested plant extracts against *P. aeruginosa* infections, especially those with multi-drug resistance [57]. In this concern clove ethanol extract showed inhibitory activity against six food associated bacteria with growth inhibitory zone diameter ranged between 25 and 32mm. The highest inhibition zone diameter (32 mm) was against *E. coli* followed by *S. aureus* (31mm) and *B. subtilis* (30mm). Equal zones of inhibition (28 mm) were recorded against *B. megaterium* and *B. sphaericus*, however the minimum inhibitory activity was recorded against *B. polymyxa* [58, 59]. This antibacterial activity is attributed to its eugenol (2-methoxy -4 allyl-phenol) content [60]. As GC-MS analysis of the clove oil extract has shown eugenol acetate, eugenol and caryo-phyllene as the major constituents, the latter two are known to possess antibacterial and antifungal properties [61, 62]. High tannin content (10-19%) in clove also provides additional antimicrobial activity [63]. Eucalyptus ethanol extract in conc. 100 mg/ml showed high antibacterial activity against 61 *P. aeruginosa* strains with growth inhibitory zones ranged from 15.09-18.35mm. However in concentration of 40 mg/ml moderate activity with inhibition zone 9.4-10.2 mm was recorded against only 24 isolated strains. In this concern significant growth inhibition for *S. aureus* and *E. coli* was induced by. *E. globulus* and *E. camaldulensise* essential oils [64, 65]. Terpineol is active component represented in essential oils of various eucalyptus species was assumed to be the main contributor for this antibacterial activity [13, 64]. E. globulus leaf extract exhibited antibacterial activity against *S. aureus, S. pyogenes, S. pneumoniae* and H. influenza isolated from human respiratory tract spacimens [66]. The authors regarded this activity to eucalyptus oil as active constituent in this tested plant extract, confirming to this, Pharmacopoeial-grade established that dried eucalyptus leaf must contain a minimum of 2.0% v/w volatile oil composed mainly of 1,8-cineole. Confirming to our results the essential oil of *E. globulus* and other oils of various *E.* species has been showen antibacterial activity against different bacterial strains [67], particularly with the most potent inhibitory activity on *P. aeruginosa* growth.

Hibiscus ethanol extract in concentration of 100 mg/ml gave intermediate antibacterial activity against only 7 *P. aeruginosa* isolated strains with inhibitory zones ranged from 9.4-10.8 mm. However the lowered concentrations lost this activity. Similar findings reaveled that *P. aeruginosa* was highly sensitive to *Hibiscus sabdariffa* ethanol extract [68] in concentration of 100mg/ml. In the same pattern methanol extract of *H. sabdariffa* leaves at concentration of 2 mg /disc inhibited both Gram-positive and Gram-negative bacteria of *E. coli*, *P. aeruginosa* and *S. choleraesuis* [69]. Mounnissamy *et al.* [70] assumed the antibacterial activity of *H. sabdariffa* to its content of protocatechuic acid which was found to have *In vitro* inhibitory activity on the growth of methicillin resistant *S. aureus* (MRSA), *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* [71]. Hibiscus may also exhibit bacteriocidal properties which is contributed mainly to its polyphenolic compounds especially gossypetin [70].

CONCLUSION

- The incidence of antimicrobial sensitivity for *P. aeruginosa* was varied for each individual antimicrobial depending on the isolate strain and source of isolation.
- Each of Clove and Eucalyptus ethanol extracts (100 mg/ml) have great potential antibacterial activity against *P. aeruginosa*, however Hibiscus ethanol extract exhibits more lesser activity than them.

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