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Effect of Temperature on the antibiotic-resistance of Proteus spp clinical Isolates

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Abstract : 503 urine samples were collected from patients suffering from urinary tract infections (UTI), chronic otitis media, wounds and burns. 68 isolates of P.mirabilis and P.vulgaris of 13.5% were used. Depending on their morphological properties and biochemical tests, the distribution of these isolates was 31 UTI samples out of 262, 15 otitis samples out of 79, 9 wound samples out of 77, and 13 burns samples out of 85. 60 isolates (88.2%) were P. mirabilis and 8 isolates (11.8%) were P. vulgaris. The other species of Proteus did not appear in the studied samples. The antibiotic sensitivity of the isolates was tested against twenty-two antibiotics, the most isolates showed high resistance. The impinem, meropenem, siftrixone, cifotaxime, amikacin, gentamicin, and ciprofloxacin were found to be more effective. The minimum inhibitory concentrations of isolates are high. The effect of temperature on Proteus spp. antibiotic resistance is studied. The temperature at 43 OC has a good effect in decreasing the bacterial resistance to the antibiotic.

Keyword: Bacteria, urinary tract infection,ear, wound and burn Infection, antibiotic sensitivity,temperature.

Introduction

Proteus bacteria are normal flora of the human gut components. They represent one of the most nurses' opportunism, because of the multiple resistances to antibiotics are the causes of major and common in causing injuries in male children and in adults of both sexes, including burns, wounds, middle ear, urinary tract, meningitis innewborn children, catarrh, sore skin and eye and diarrhoea. Members of this genus cause of many diseases to humans and animals, *P. mrabilis* causes many infections acquired from the hospital, as it comes in second place after the type *E.coli* in bringing acquired U.T.Is injuries from the hospital. *P. mirabilis* isolated from different pathological cases are more isolated types (61.5%), followed *P. vulgaris* (30.5%) and then *P. penneri* (8%)[1].These bacteria have a high the ability of urea analysis and it is distinct from the rest of the intestinal family members, as this has five main types are: *P. mirabilis, P. vulgaris, P. myxofaciens, P. penneri*. This bacteria has several ferocity factors that help with the disease, which include *Flagella, Fimbria, Protease* and *Hemolysin*, and *Urase* be causing the kidney stone and has a relationship in *Septicemia* as it increases the proportion of ammonia in the blood and prevents activation of the complement fourth C4.As well as cause a high pH in the urine, leading to decomposition of white blood cells present in the area of the injury, the usability of adhesion in the epithelial cells, and Invasiveness and *lipopolysaccharide* called *Endotoxin*. *Proteus* also has the ability to form Biofilm and excellence in Swarming. All of these factors make *Proteus* bacteria are able to overcome the various means of defence, which is owned by the host [2]. The expansion of the indiscriminate use of antibiotics has led to the emergence of some breeds mutant of these bacteria, which are

resistant to most antibiotics[3]. Therefore, the effectiveness of many of the antibiotics used to treat bacterial infections, including infections at *Proteus* has become quite specific because of bacterial resistance.

Materials and Methods

503 samples of urine of patients with urinary tract infections and inflammation of the middle ear and wounds and burns were collected for both sexes and various ages, and then transferred the samples to the laboratory for testing. *P. mirabilis* and *P.vulgaris* bacteria causing infections (urinary tract, the middle ear, wounds and burns) have been isolated after the samples are lying on the blood agar base (Oxoid) and MacConkey agar (Himedia) by planning technique then was diagnosed based on phenotypic attributes and biochemical tests [4].Isolates susceptibility or their sensitive on 22 antibiotics equipped by Bioanalyse company-Turkey is tested[5]. The impact of the change in the temperature-resistant bacterial by vaccination 10 ml of nutrient broth in a single colony of the isolates of the bacteria to be tested and incubated for 24 hours at a temperature of 37 ^{o}C.Then 0.1 ml of the cultivated bacterial added to 10 ml of nutrient broth and incubated at temperatures of 37 ^{o}C, 43 ^{o}C and 45 ^{o}C for 18 hours in an incubator (100 rpm). After incubation, the process of dilution was conducted and 0.1 ml of the last three dilutions has been distributed in nutrient agar dishes using spreader, then 100 bacterial colonies treated by heat and transferred to the nutrient agar which represents the master plate and incubated at 37 °C for 24 hours. Isolates resistant to antibiotics are tested through its transfer to nutrient agar and then antibiotics tablets are putted. Inhibition in mm was measured and compared with the inhibition zone diameters with peers to investigate the effect of changing the temperature on bacterial resistance [5].

Results and Discussion

Results showed that the total isolation of *Proteus* bacteria ratios of 13.5% and 88.2% of *P.mirabilis* while 11.8% of *P.vulgaris* as shown in Table 1. While, the ratio was 14% for *P.mirabilis* and 10% for *P.vulgaris*[6]. The isolation ratios in different patient body positions were 78.3% and 16.2%, respectively[7]. *Proteus spp*.is common in the incidence of urinary tract infections and comes after *E.coli* and complexities associated with may be similar to *P.aeruginosa* in the infected tissues compared to other bacterial species[8].

Table 1. Number and percentage of isolates of *Proteus* bacteria and distributed according to injury location

Isolates Source	No. of Sample	Bacterial growth (+)	Bacterial growth (-)	*P. mirabilis*	*P. vulgaris*	Total No. of Isolates	%
Urine	262	224	38	27	4	31	13.8
Ear	79	67	12	14	1	15	22.4
Wounds	77	56	21	7	2	9	16.0
Burns	85	71	14	12	1	13	18.3
Total	503	418	85	60	8	68	16.3

The total isolation ratio of the ear was 22.4% (*P.mirabilis* (20.9%) and *P.vulgaris* (1.5%)). The total isolation ratio of the wounds was 16.0% (*P.mirabilis* (12.5%) and *P.vulgaris* (3.6%)). The total isolation ratio of the burns was 18.3% (*P.mirabilis* (16.9%) and *P.vulgaris* (1.4%)). The study results showed that the highest percentage of resistant isolates were 100% for *Carindacillin, ampicillin, tetracycline*, and *erythromycin*, which is identical with[9], and 82.4% for *Trimethoprim*. The isolates were classified by phenotypic characteristics and biochemical tests as shown in Table 2.Table 3 showed antibiotic susceptibility test.

Table 2.Microscopic and biochemical tests *Proteus* isolates from different cases of the diseases

Type of test	***P. mirabilis***	***P.vulgaris***
Gram stain	G-ve	G-ve
Catalase	+	+
Swarming	+	+
Oxidase	-	-
Lipase	+	+
H2S	+	+
Gelatin liquefaction	+	+
Motility	+	+
Indole	-	+
Methyl red	+	+
VogesProskaur	+/-	-
Citrate utilization	+/-	+/-
Creatinine	+	+
Kligler iron agar	Alkaline/Acidic	Alkaline/Acidic
Orinthindecarboxelase	+	-
Fructose	+	+
Lactose	-	-
Sucrose	+/-	-
Maltosum	-	+
Mannose	+/-	-
Xylose	+/-	+
Trehalose	+/-	+/-
Mannitol	+	+/-
Phenylalanine	+	-
Arginine- (Carboxylic acid)	-	+
Bacterial growth at 43^{o}C	+	+

+: Positive result; −: Negative result

Table 3. Proteus spp. bacteria resistant in cases of a different diseases

Carbon Celine	*Cefotaxime*	*Amikacin*	*Ampicillin*	*Tetracycline*	*Saybrovluxasin*	*Siftarickson*	*Svetozdam*	*Gentamicin*	*Erythromycin*	*Chloramphenicol*	*Aztreonam*	*Amoxicillin*	*Tekarcelin*	*Levofloxacin*	*Cefepime*	*Traymthbrim*	*Norfloxacin*	*Rifampicin*	*Kanamycin*	*Meropenem*	*Imipenem*	*Isolate*
																						P.mirabilis
R	S	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	S	R	1
R	R	S	R	R	I	S	I	R	R	R	S	R	R	S	S	R	R	R	R	S	S	2
R	S	R	R	R	I	S	I	R	R	R	S	R	R	R	S	R	S	S	R	S	S	3
R	I	S	R	R	S	S	S	S	R	R	S	R	R	R	S	R	S	R	R	S	S	4
R	R	R	R	R	R	I	R	R	R	R	S	S	R	R	S	R	S	S	R	S	S	5
R	S	S	R	R	S	R	R	S	R	R	S	R	S	S	S	R	S	S	R	S	S	6
R	S	R	R	R	S	R	R	R	R	R	S	I	R	R	S	R	S	S	R	S	S	7
R	S	I	R	R	I	S	R	R	R	R	S	R	R	R	S	R	R	R	R	S	S	8
R	S	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	S	S	R	S	S	9
R	R	S	R	R	R	I	S	R	R	R	S	R	R	R	S	R	R	S	R	S	S	10
R	S	I	R	R	S	S	R	S	R	R	R	R	R	S	S	R	R	R	R	S	S	11
R	R	R	R	R	S	S	R	S	R	R	R	I	R	R	S	R	S	R	R	S	S	12
R	S	S	R	R	R	S	S	R	R	R	R	R	R	S	R	R	R	S	R	S	S	13
R	R	R	R	R	S	S	R	S	R	R	S	S	R	S	I	R	R	R	S	S	S	14
R	S	R	R	R	R	S	S	R	R	R	S	R	R	R	R	S	S	S	R	S	S	15
R	S	R	R	R	R	S	R	R	R	R	S	S	R	S	R	R	R	S	R	S	S	16
R	S	S	R	R	S	S	R	R	R	R	R	R	R	S	S	R	R	R	R	S	S	17
R	R	R	R	R	R	S	R	R	R	R	R	R	R	S	R	R	R	R	S	S	S	18
R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S	S	19
R	S	S	R	R	S	S	S	R	R	R	S	R	R	R	S	R	S	S	R	S	S	20
R	R	S	R	R	R	S	R	R	R	R	R	R	R	S	S	R	S	S	R	S	S	21
R	S	S	R	R	R	S	R	S	R	R	S	R	R	S	S	R	R	R	R	S	S	22
R	S	R	R	R	R	S	R	S	R	R	R	R	S	S	R	R	R	R	R	S	S	23
R	R	S	R	R	S	R	S	S	R	R	R	R	R	S	R	R	R	R	R	S	S	24
R	R	S	R	R	S	I	R	S	R	R	S	R	R	S	R	R	R	S	R	S	S	25
R	S	S	R	R	R	I	R	S	R	R	R	R	R	S	S	R	R	S	R	S	S	26
R	R	S	R	I	S	R	R	R	R	R	S	R	R	R	R	R	S	S	R	S	S	27
R	R	S	R	R	R	R	R	R	R	R	S	R	R	S	S	R	R	R	R	S	S	28
R	S	R	R	R	S	S	I	S	R	R	R	R	R	S	R	S	S	S	R	S	S	29
R	S	R	R	R	R	I	I	S	R	R	S	I	R	S	R	R	R	R	R	R	R	30
R	S	S	R	R	R	S	R	I	R	R	S	R	R	S	R	R	R	R	R	S	S	31
R	R	R	R	R	R	R	R	S	R	I	S	R	R	R	R	R	R	R	R	S	S	32
R	S	S	R	R	S	S	R	S	R	R	R	R	R	S	R	R	R	R	R	S	S	33
R	S	S	R	R	R	S	R	S	R	R	R	R	R	S	R	R	R	R	R	S	S	34
R	R	S	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	S	S	35
R	R	S	R	R	S	S	R	S	R	I	R	R	R	S	R	R	S	R	R	S	S	36
R	R	S	R	R	R	S	R	R	R	R	R	R	R	S	R	R	R	S	R	S	S	37
R	R	S	R	R	R	S	R	S	R	R	R	R	R	S	R	S	S	S	R	S	S	38
R	R	S	R	R	S	R	R	S	R	R	R	R	R	R	R	R	S	R	S	S	S	39
R	*R*	*S*	*R*	*R*	*S*	*I*	*R*	*S*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*R*	*S*	*S*	40

R	R	I	R	R	R	S	R	R	R	R	I	R	R	S	S	R	R	R	R	S	S	41
R	R	S	R	I	R	S	R	S	R	R	R	I	R	S	R	S	S	R	R	S	S	42
R	I	S	R	R	S	I	R	S	R	R	R	R	R	S	S	R	I	R	R	S	S	43
R	R	S	R	R	S	S	R	S	R	R	R	R	R	S	R	R	S	S	R	S	S	44
R	R	S	R	R	R	R	S	S	R	R	R	R	S	R	S	S	S	S	R	S	S	45
R	R	R	R	R	R	S	R	R	R	S	R	S	R	R	R	R	R	S	R	S	S	46
R	S	S	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	S	S	47
R	S	S	R	R	R	S	R	S	R	R	R	R	R	R	S	R	I	S	R	S	S	48
R	S	S	R	R	R	S	R	S	R	R	R	R	R	S	S	R	R	S	R	S	S	49
R	R	S	R	R	S	S	S	S	R	R	R	R	R	R	R	R	S	S	R	S	S	50
R	R	S	R	R	R	S	R	I	R	R	R	R	R	R	R	R	S	S	R	S	S	51
R	R	S	R	R	S	S	R	R	R	R	R	I	R	R	S	R	R	I	R	S	S	52
R	I	S	R	R	R	S	R	S	R	R	R	R	R	R	S	R	R	S	R	S	S	53
R	S	S	R	R	S	R	S	S	R	R	S	R	R	R	S	R	S	R	R	S	S	54
R	R	S	R	R	R	S	R	R	R	R	R	R	I	R	S	R	R	R	R	S	S	55
R	S	S	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	S	R	S	S	56
R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	S	S	R	S	S	57
R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	S	S	58
R	S	S	R	R	S	S	S	S	R	S	R	R	R	S	R	S	S	S	R	S	S	59
R	S	R	R	R	I	I	I	R	R	R	R	I	S	S	S	S	R	R	R	S	S	60
																						P.vulgaris
R	R	S	R	I	S	S	R	I	R	S	R	S	R	R	S	S	S	S	R	S	S	61
R	R	S	R	R	R	S	S	S	R	I	I	R	R	S	R	R	R	R	S	S	S	62
S	S	R	R	R	S	R	R	S	R	S	R	R	R	S	S	R	S	R	R	S	S	63
R	S	S	R	R	I	S	S	S	R	S	S	S	R	R	R	S	R	R	S	S	S	64
R	S	S	S	R	S	S	R	S	R	S	S	R	R	S	S	R	S	S	R	S	S	65
R	R	S	R	R	S	S	S	S	R	I	I	R	R	S	S	S	S	S	R	S	S	66
R	R	S	R	R	R	S	R	R	R	S	S	R	S	S	S	R	R	S	R	S	S	67
R	S	S	R	R	S	S	R	S	R	S	R	R	R	R	S	S	S	S	S	S	S	68

R: Resistance, S: Sensitive, I: Medium

The results showed that the highest ratios of resistance found from some *β-Lactam*whichit's working to discourage the construction of the cell wall being involved in making the peptidoglycan layer as well as the absence of an anti-penicillin or decrease in the bacterial cell wall [10].The percentage ratio for *amoxicillin* and *ticarcillin* found to be 80.9 and 92.6, respectively. Kahlmeter, 2003 found that the ratio of resistance to gentamicin is 1.6% of the isolates caused *Proteus* in occur of urinary tract infection while *Proteus* resistance in the current study found 42.6%[11].The study results showed that the best antibiotics in their impact on the *Proteus* isolate are *quinolones* group of isolates resistant to *ciprofloxacin* (45.6%), while the *norfloxacin* was (55.9%). Mirobenem and alambinm belonging to the carbinm group are the most effective against *Proteus* have a ratio of 100% and 98.5% respectively. While, isolate resistant of *Svetozdam*, *altramthberam*, *cefotaxime*, and *gentamicin* has a ratio of 72.0%, 82.4%, 42.6%, 42.6%, respectively. The use of *meropinem*, *impinem*, *amikacin*, *gentamicin* and *ciprofloxacin* were effective against most of the isolates under study characterized by multi-resistant to antibiotics and this corresponds to Zerovs and Foch, 2003 [12]. Current results showed that the percentage of isolates resistant to *amikacin* and *ciprofloxacin* were 27.9% and 45.6% respectively. *amikacin* and *ciprofloxacin* are the best antibiotics for any body infections, especially urinary tract infections caused by bacteria. Some *Proteus spp*. isolates were resistant to most antibiotics. Most *Pseudomonas spp*, *Enterobacteriacea*, and *Proteus spp.* showing complications in the urinary tract inflammation in terms of treatment and prolonging the treatment period. The *P. mirabilis* isolated from urinary tract infections were resistant to 19 antibiotics out of 22, perhaps the reason for the multiplicity of bacterial resistance movement of

genetic material such as *R-Plasmid*, *Transposon* or DNA to other sensitive to these antibiotics. All isolates were tested for antibiotics, as the method turbidity bacterial growth is used in the liquid and subculture in order to determine the presence or absence of bacterial growth, as identified less concentration of antibiotics as shown in Table 4. Minimum inhibitory concentrations of antibiotics under study have high values. In this study, minimum inhibitory concentrations of antibiotics have high values. As it reached 128 <-64 micrograms / ml for some isolates and 128 <-16 for some of the other which shows resistance to these antibiotics has been largely due to the development of the capabilities of bacteria in repelling treatments and the use of a broad and random to antibiotics. The rest of the antibiotics were less, *sifterickson* and *saybrovluxasin* are the most efficient and lowest inhibitor concentrations ranged between 0.5-32 and 0.5-64, respectively. Guven, 2004 found that the value of MIC to Ciprofloxacin was 2.1 micrograms/ml of Proteus isolates of biofilms formed on medical therapeutic equipment [13].

Table 4. Minimum inhibitory concentrations of antibiotics for bacteria *Proteus spp.*

Antibiotic	***Proteus spp. %***	***Proteus spp.*MICs(µg/ml)**
Tekarcelin	92.6	128 ->16
Levofloxacin	42.6	128 -0.5
Saybrovluxasin	45.6	64 -0.5
Rifampicin	45.6	128 -1
Tetracycline	100	128->64
Siftarickson	22.0	32 -0.5
Cefepime	41.1	128 ->4
Meropenem	0.0	8 -0.5
Amikacin	27.2	0.5-32
Ampicillin	100	128 ->32

Some of the isolates showed resistance to *ravambisn* and *amikacin*. The MIC values ranged between 128-1 and 0.5-32 micrograms / ml, respectively, the reason for increased resistance to two antibiotics in the current study may be due to increased use of antibiotics for the treatment of urinary tract infection.*P.mirabilis* and *P.vulgaris* were resistant to all antibiotics used in this study at 37 °C, while the variation observed the isolates resistant to antibiotics when raising the temperature at 40 and 43 m, as shown in Table 5. *P.mirabilis1* was resistant to *sifitrixone* at 37 °C. While, *P.mirabilis1* shifted from resistance to sensitive and at inhibition of 23 mm diameter at 40 °C.Inhibition diameter increases to 31 mm when the temperature of the incubator rise to 43 m, and this is evidence that the sensitivity *P.mirabilis1* to antibiotics.*P. mirabilis* 1 was also resisting to *ampicillin* and *amikacin* antibiotics until reaching a temperature of 40 °C. This can be seen the same gradient in the antibiotic resistance of *sifitrixone* for isolates *P.mirabilis* 32 and *P.vulgaris* 63 as they both strains resistant to the anti at a temperature of 37 °C, then became the strains are sensitive to the anti at a temperature of 40 °C to 43 °C, and can be inferred that the change *Proteus* growth temperatures affect negatively when lifting in the wall of bacteria and function of the fundamental.*P.vulgaris*63 have been highly resistant to *ampicillin* and *amikacin* as strains continued to resist these two anti-especially *ampicillin*, although raising the growth temperature to 43 °C. The resistance to this anti continues to increase, especially in recent times, as well as the *Proteus* bacteria resistant is one of the natural resistance of these bacteria [14] as shown in Table 5.The bacteria continued to resistance to these antibiotics as a mechanical resistance at the temperature 40 °C for *ampicillin* and *amikacin* and at 43 °C for the *ampicillin*.

Table 5. Effect of temperature change in the resistance of bacteria to antibiotics

Isolate	Temperature ^{O}C	Antibiotic and diameter (mm)		
		Sifitrixone	***Ampicillin***	***Amikacin***
P.mirabilis1	37	R(20)	R(6)	R(11)
	40	S(23)	R(11)	R(9)
	43	S(31)	S(30)	S(27)
P.mirabilis32	37	R(10)	R(2)	R(0)
	40	S(15)	R(3)	R(0)
	43	S(30)	S(35)	S(29)
***P.vulgaris*63**	37	R(11)	R(0)	R(0)
	40	S(20)	R(2)	R(0)
	43	S(26)	R(6)	S(31)

Conclusions

The current study showed that the temperature 43°C mediated between multiple resistance and sensitive to *Proteus* antibiotics for *amikacin*. All isolates continued to resist this anti in temperature 37 and 40 °C, while it was sensitive to *amikacin* at 43 °C. The reason may refer to resist the wall of the bacterial cell in the change in the temperature of the incubator 37 and 40 °C and bacteria, it cannot grow well or build a wall in an integrated manner at a temperature of 43 °C, allowing to anti the access through the wall of bacteria and destroy it.

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