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Antipseudomonal property of honey and aminoglycosides

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Abstract: *Pseudomonas aeruginosa* has an ability to rapidly develop resistance to most antimicrobial compounds, and to check this ability. The isolates were collected from different pathological human sources and tested for their sensitivity to aminoglycoside antibiotic and to honey, a natural product that is generating renewed interest for its therapeutic application using Kirby Bauer disc diffusion technique. Aminoglycoside antibiotic which is normally active against gram-negative bacteria was used alongside honey. The 29 isolates of *P. aeruginosa* showed 100% sensitivity to honey tested in their undiluted form. This was not the case with gentamicin (10µg) and amikacin (30 µg), both of which varied in their antipseudomonal activity, like even 1:2 aqueous dilution of honey appreciably inhibited pseudomonal isolates than either of the two aminoglycoside antibiotic. Honey is therefore suggested as an effective natural product in overcoming the widespread antibiotic resistance of *P. aeruginosa*.

Keywords: Amikacin, Antipseudomonal activity, Gentamicin, Honey

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen that causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections, particularly in victims of severe burns, and in cancer and AIDS patients who are immuno-suppressed (Cachia and Hodges, 2003). *P. aeruginosa* is not particularly distinctive as a pseudomonad, but there are a few characteristics that are noteworthy and relate to its pathogenesis. *P. aeruginosa* possesses the metabolic versatility for which pseudomonads are so renowned. *P. aeruginosa* produces two types of soluble pigments, pyocyanin and (fluorescent) pyoverdin. The latter is produced abundantly in media of low-iron content, and could function in iron metabolism in the bacterium (Todar, 2004). Pyocyanin (from "pyocyaneus") refers to "blue pus" which is a characteristic of suppurative infections caused by *P. aeruginosa*. *P. aeruginosa* is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen. Comparatively in the aminoglycosides, gentamicin, in combination with vancomycin or penicillin, provides a good remedy, also gentamicin was observed in an in vitro experiment to effectively inhibit gram-negative bacterial infections particularly *P. aeruginosa* ((Klika and Goodman,1982; Osman *et al.,* 2003). Similarly, honey was reported to be effective against a wide range of medically important bacteria and even higher fungi (Tysett *et al.,* 1993, Molan, 1999).

Honey is produced from many floral sources and its antimicrobial activity varies markedly with its origin and processing (Willix *et al.,* 1992; Cooper and Molan, 1999; Cooper *et al.,* 2002). This variation can be due to difference in the enzymatic action and in the presence of additional antibacterial components derived from the floral source (Irish *et al.,* 2011). Due to emergence of antibiotic resistant strains, *P. aeruginosa* isolated from different anatomical sites were evaluated for their antibacterial action using honey and aminoglycosides.

MATERIALS AND METHODS

Twenty nine isolates of *P. aeruginosa* from various pathological sources (Table 1) were obtained on sterile nutrient agar (Hi-media) slants from hospitals in and around Vapi area, Gujarat. They were re-isolated on king's agar and subjected to conventional tests (Cowan, 1974) and then preserved on fresh nutrient agar slants in a refrigerator at 4^0C. The honey used in the present study was from Pardeshi babul and Neem forest of Kachchh and Banaskantha districts, which was used in undiluted form and also as 1:2 aqueous dilutions against each isolates of *P. aeruginosa*. The aminoglycoside antibiotic (Hi-media) was also used alongside honey against each *P. aeruginosa* isolate.

The susceptibility pattern of each pseudomonal isolate was obtained following the standard single disc diffusion method developed by Bauer *et al*. (1966) against undiluted honey and its 1:2 aq. dilution and aminoglycoside. The results were interpreted according

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to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and the organisms were scored as either sensitive or resistant.

RESULTS

A total of 29 isolates were obtained from 300 clinical samples collected from patients having pyogenic infection, amongst the clinical isolates, 22 (75.9%) were from ear swab, 4 (13.8%) from wound swab, 2 (6.9%) from pus swab and 1 (3.4 %) from burn wound.

Table 1. Pathological sources of *Pseudomonas aeruginosa*.

Pathological source	No. of isolates
Wound swab	4
Ear swab	22
Burn wound	1
Pus swab	2

Screening for Multi Drug Resistant (MDR) isolates showed that 27/29 (93%) isolates were MDR strains showing resistance to at least 3 antibiotics and to third generation cephalosporin. Antibiotic susceptibility testing of the isolates showed varying degree of sensitivity to the antibiotics tested. Highest resistance was seen for tetracycline and cefuroxime (86.2%), followed by clindamycin (75.9%) and ampicillin/ sulbactum (72.4%) (Fig. 1).

The undiluted honey (neat honey) used for each pseudomonal isolate produced zones of growth inhibition ranging from 14.4 to 25.4 mm, indicating 100% sensitivity of all isolates of *P. aeruginosa* against neat honey. However, gentamicin, amikacin and the 1.2 aq. dilutions of the honey sample varied in their growth inhibition (Table 2). In the honey sample, only one isolate failed to be inhibited (3.4% resistance) by 1.2 aq. dilutions, while

Table 2. Antibacterial activity of honey and aminoglycoside against clinical isolates of *P. aeruginosa.*

Isolate	Honey		Gentamicin	Amikacin
Source	0	1:2	10 µg/ml	30 µg/ml
	Diameter of zone of inhibition (mm)			
Wound swab	15.7	10.1	11.4	14.4
Ear swab	20.2	15.3	9.5	8.8
Ear swab	17.0	11.0	9.7	9.2
Ear swab	20.1	15.0	9.4	10.7
Pus swab	14.4	10.4	11.9	11.9
Ear swab	21.7	16.2	13.8	14.7
Ear swab	22.5	13.2	14.5	13.1
Ear swab	15.2	9.4	8.2	8.7
Ear swab	16.4	-	11.8	7.9
Ear swab	22.5	16.7	14.0	15.3
Ear swab	23.7	13.3	14.9	14.0
Ear swab	23.4	14.2	8.7	9.0
Wound swab	21.4	10.0	5.5	6.6
Ear swab	20.2	10.1	6.3	5.3
Ear swab	17.3	9.4	-	-
Ear swab	16.7	9.2	8.7	7.7
Wound swab	22.8	11.8	7.7	9.3
Ear swab	24.0	17.2	9.4	9.4
Ear swab	24.4	16.8	11.8	11.7
Burn wound	22.9	13.6	12.0	13.6
Ear swab	17.7	11.6	15.4	14.7
Ear swab	19.6	12.6	6.9	7.6
Ear swab	24.7	16.3	-	11.2
Ear swab	25.4	15.7	16.8	11.7
Wound swab	23.6	13.8	13.3	14.5
Ear swab	22.4	14.9	13.4	9.1
Ear swab	19.8	11.0	11.9	13.8
Pus swab	19.8	10.8	7.7	-
Ear swab	21.5	11.7	10.6	12.5

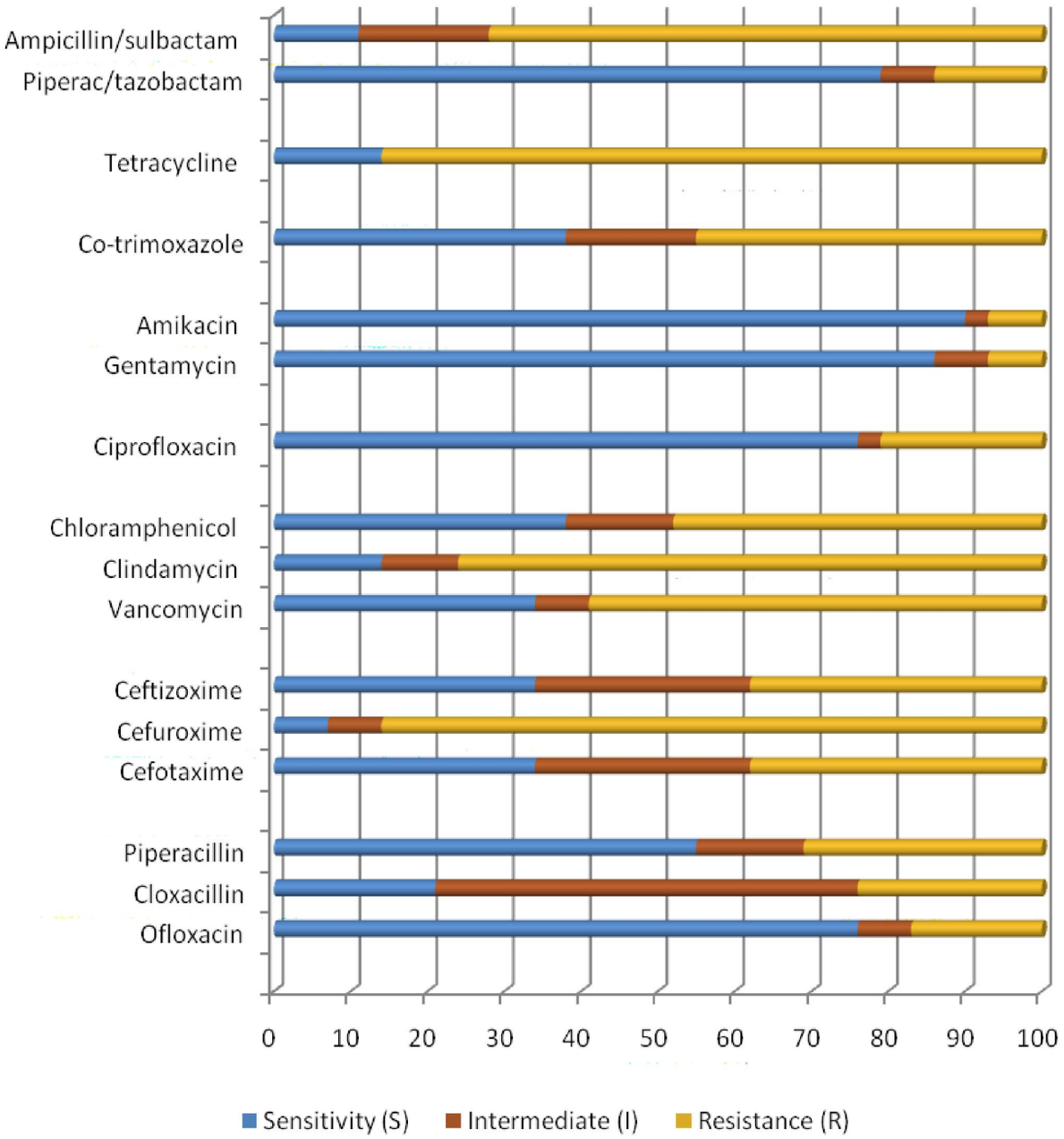

Fig 1. *Antibiotic susceptibility pattern of P.aeruginosa isolated from various pathological source.*

gentamicin and amikacin failed to inhibit 2 of the 29 isolates (6.9%) (Table 2).

DISCUSSION

P. aeruginosa has long been recognized as a major burn pathogen and is the predominant cause of fatal burn wound sepsis (Artz *et al.*, 1969; Hummel *et al.*, 1970; Teplitz, 1979; Mohr *et al.*, 2004). Interest has increased in the alternative natural therapies with the isolation of multidrug resistant strains, in that honey has received renewed recognition as burn-wound healers. This natural product was found to be individually effective against bacteria, although the aminoglycoside used alongside was reported to be that effective.

In the present study the results were in agreement with the study of Tan *et al.* (2009) who reported that Indian origin honey have the activity against *P. aeruginosa* isolates compared to honey like Tualang and Manuka. Lusby *et al.* (2005) also reported that local honey used have the same antibactericidal activity as compared with other pharmaceutical honeys, this is evident in the 100% sensitivity of the pseudomonal isolates to the undiluted

Table 3. Relative percentage resistance of clinical isolates of *P. aeruginosa* to honey, gentamicin and amikacin.

Honey		Gentamicin	Amikacin
0	1:2	10 µg/ml	30 µg/ml
0%	3.4%	6.9%	6.9%

0 = undiluted honey, 0% = no resistant isolate

stock of the honey sample tested. This activity was also shared by the 1:2 aq. dilution of the honey and gentamicin (10 µg) and amikacin (30 µg) with a few numbers of resistant isolates which is in partial agreement with Adeleke *et al*. (2006). Richard *et al*. (1988) in his study found that aminoglycoside particularly gentamicin and amikacin showed no and little bactericidal activity respectively against the isolates which is in partial agreement with our study, whereas Basson and Grobler (2008) found no such antimicrocidal activity in honey from South Africa, the native place. It has been found that honey is not only sound as a topical healing agent but also has antibacterial and antifungal properties and promotes wound healing with less scar formation (Subrahmanyam *et al.,* 2001; Osman *et al.,* 2003). Notably, the fact that the strains of *P. aeruginosa* tested came from different human pathological sources lends credence to honey's therapeutic value.

Conclusion

In conclusion, honey a natural, non-toxic and inexpensive product has the activity against *P. aeruginosa* isolated from different human pathological sources indicating the medicinal importance of Indian honey against these isolates.

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