http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (10): 1719-1723, 2000

Antibiogram Sensitivity of Bacterial Organisms Identified from Surgical and Non-surgical Wounds of Animal

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Abstract: Eight different antibiotics were used against bacterial species. The antibiotics were tetracycline, ampicillin, gentamicin, kanamycin, neomycin, baquiloprim sulphadimidine, chloramphenicol and sulphamethoxazole trimethoprim. The species and their percent of sensitivity to tetracycline were: *Streptococcus pyogenes* (80%), *Streptococcus uberis* (73.3%), *Staphylococcus aureus* (80%), *Corynebacterium diphtheriae* (73.3%) and *Micrococcus luteus* (73,3%). Whereas *Streptococcus intermedius* (80%), *Pseudomonas aeruginosa* (73.3%) and *Stomatococcus mucilaginous* (80%) were highly sensitive to ampicillin only. The species *Streptococcus pyogenes* (73.3%). *Proteus vulgaris* (100%), *Pseudomonas aeruginosa* (86.6%), *Micrococcus luteus* (93.3%) and *Stomatococcus mucilaginous* (86.6%) showed sensitivity to chloramphenicol. Other species recorded highly sensitive to Sulphamethoxazole trimethoprim were: *Streptococcus pyogenes* (100%). *Streptococcus uberis* (80%), *Staphylococcus aureus* (73.3%), *Corynebacterium diphtheriae* (73.3%), *Corynebacterium pyogenes* (73.3%), *Proteus vulgaris* 173.3%), *Pseudomonas aeruginosa* (80%), *Micrococcus luteus* (86.6%) and *Stomatococcus mucilaginous* (100%) respectively.

Key words: Animal wounds, bacterial organisms, antibiogram sensitivity

Introduction

This kind of race in between pathogenic bacterial species and drug companies is still going on since last few decades. The battle between bacteria and their susceptibility to drugs is yet problematical among public, investigators and drug companies to find out an effective drugs against a variety of bacterial organisms. Previously, a lot of work has been taken-up allover the world and many recommendations have already been made by different workers on the susceptibly of the organisms to various antibiotics (Mathews *et al*., 1992; De Aguayo *et al*., 1992; Singh *et al*., 1992). Singh *et al*. (1992) studied 25 pus swabs from non-surgical wounds and recognized the *Proteus vulgaris, Staphylococcus aureus, Bacillus* sp. and *Escherichia coil* in 14, 21, 14 and 10 samples respectively. The sensitivity of these isolates to different antibiotics was assessed using the disc diffusion technique. Most of the isolates were found resistant to erythromycin, ledermycin, tetracycline, vibramycine, cloxacillin, chloramphenicol, penicillin, gentamicin and kanamycin. Keeping in view the susceptibility and resistance of bacterial species to antibiotics, the present study was planed to demonstrate the sensitivity of the organisms to antibiotics those cause wounds in skins and hides of animals.

Materials and Methods

One hundred samples were collected from surgical and nonsurgical wounds located on the body surface of sheep, goats, cattle and buffaloes. The samples were obtained from Veterinary Clinic Sindh Agriculture University, Tando Jam and Civil Veterinary Hospital Heraabad, Hyderabad. All samples were processed and examined at Central Veterinary Diagnostic Laboratory, Tando Jam for isolation of bacterial organisms. Before collection, the surroundings of the severely injured, operated and or un-opened wounds were cleaned with antiseptic (spirit) and then swabs were taken by removal of extraneous contaminant organisms. But in the case of un-opened wounds (abscesses) the surface of abscesses was cleaned properly with antiseptic and then incision was made and samples were collected into bijou's bottle as well as by cotton swabs. After collection and before processing the samples, all glassware, new and used were kept in 1% HCI solution overnight. The glassware were removed from solution and washed well with distilled water for several times then dried in oven at 65°C. The sterilization was carried-out in hot air oven at 180°C for one and half an hour (Cruickshank, 1970). The media were prepared and inoculated by wound samples for identification of bacterial species as described by Cruickshank (1970). Both the media, solid and liquid were used. In solid: nutrient, blood and MacConkey's agars and while in broth: nutrient broth was prepared, cultured and specific colony characteristics of the species were recorded. A subculture was made and a pure colony from dish was picked-up and smeared on a cleaned glass slide and stained by Gram's method of staining and all morphological characteristics recommended for identification were observed as described by Cruickshank (1970). A few biochemical tests were also carried-out to confirm the specific chemical characteristics of the organism. For this purpose, oxidase, coagulase, indole, Voges Proskauer, urease, methyl red, gelatin liquefaction, Sirnmon's citrate, H_2S production, catalase and TSI tests were conducted (Cruickshank, 1970). For sugar fermentation properties of each species as a tool for their identification, eight different sugars of 1% were prepared and used for each isolated bacterium as prescribed by Cruickshank (1970). The sugars used were: glucose, sucrose, lactose, maltose, mannitol, inositol, arabinose and raffinose. For the sensitivity of the organisms to different antibiotics, the following discs used were Tetracycline (TC), Ampicillin (AM), Gentamicin (G), Kanamycin (K), Neomycin (N), Baquiloprim sulphadimidine (BSD), Chloramphenicol (CN), Sulphamethoxazole Trimethoprim (SXT). All discs were of 30 mg. Testing the antibiotic sensitivity of the organism by Bauer *et al*. (1966) method, the following materials were brought in use:

1. Mueller-Hinton agar plates
2. 150 × 15 mm, 4-6 mm deep medium
3. Sterile saline
4. Barium chloride standard
5. Sterile cotton wool or sterile swabs
6. Sterile forceps
7. Ruler
8. Sensitivity chart

Table 1: The antibiotic sensitivity of various organisms identified from different wound samples of domestic animals

Bacterial Species	Antibiotic Discs used Disceimmi	Zone around	Indication of sensitivity	% of sensitivity sensitivity	Degree of
Streptococcus pyogenes	tritracycline	12mm	+ + + +	80.0	Highly sensitive
	Ampicillin	10mm	+ + +	60.6	Quite sensitive
	Gentamicin	2mm	+ +	13.1	Moderately sensitive
	Kanamicin	4mm	+ +	26.6	Moderately sensitive
	Neomycin	3mm	+ +	20.0	Moderately sensitive
	Baquiloprim Sulphadimidine	0mm		0.0	Resistant
	Chloramphenicol	11mm	+ + + +	73.3	Highly sensitive
	Sulphamethoxaxole trimethoprim	15mm	+ + +	100.0	Highlysensitive
Streptococcus uberis	Tetracycline	11mm	+ + + +	73.3	Highly sensitive
	Ampicillin	13mm	+ + + +	86.6	Highly sensitive
	Gentamicin	4mm	+ +	26.6	Moderately sensitive
	Kanamycin	3mm	+ +	20.0	Moderately sensitive
	Neomycin	2mm	+	13.0	Weakly sensitive
	Baquiloprim Sulphadimidine	lmm	+	6.6	Weakly sensitive
	Chloramphenicol	10mm	+ + +	66.6	Quite sensitive
	Sulphamethoxazole trimethoprim	12mm	+ + + +	80.0	Highly sensitive
Staphylococcus aureus	Tetracycline	12mm	+ + + +	80.0	Highly sensitive
	Arnplcillin	15mm	+ + + +	100.0	Highly sensitive .
	GentamicIn	8mm	+ + +	53.3	Quite sensitive
	Kanemycin	10mm	+ + +	66.6	Quite sensitive
	Neomycin	4mm	+ +	26.6	Moderately sensitive
	Baquiloprim Sulphadimidine	10mm	+ + +	60.6	Quite sensitive
	Chloramphenicol	8mm	+ + +	53.3	Quite sensitive
	Sulphamethoxazole trimethoprim	11min	+ + + +	73.3	Highly sensitive
zitaphylococcus intermedius	Tetracycline	10mm	+ + +	66.6	Quite sensitive
	Ampicillin	12mm	+ + + +	80.0	Higi'ily sensitive
	Gentamicin	8mm	+ + +	53.3	Quite *sensitive*
	Kanamycin	9mm	+ + +	60.0	Quite sensitive
	Neomycin	8mm	+ + +	53.3	Quite sensitive
	Baquiloprim Sulphadimidine	3mm	+ +	20.0	Moderately sensitive
	Chloramphenicol	7mm	+ + +	46.6	Quite sensitive
	Sulphamethoxazole trimethoprim	10mm	+ + +	66.6	Quite sensitive
Corynebacterium diphtheriae	Tetracycline	11 mm	+ + +	73.3	Highly sensitive
	Ampicillin	5mm	+ + +	33.3	Moderately sensitive
	Gentamicin	2mm	+	13.3	Weakly sensitive
	Kanamycin	8mm	+ + +	53.3	Quite sensitive
	Neomycin	5mm	+ +	33.3	Moderately sensitive
	Baquiloprim Sulphadimidine	10mm	+ + +	66.6	Quite sensitive
	Chloramphenicol	9mm	+ + +	60.0	Quite sensitive
	Sulphamethoxazole trimethoprim	11 mm	+ + + +	73.3	Highly sensitive
Corynebacterium pyogenes	Tetracycline	12mm	+ + + +	80.0	Highly sensitive
	Ampicillin	5mm	+ +	33.3	Moderately sensitive
	Gentamicin	3mm	+ +	20.0	Moderately sensitive
	Kanamycin	4mm	+ +	26.6	Moderately sensitive
	Neomycin	4mm	+ +	26.6	Moderately sensitive
	Baquiloprim Sulphadimidine	3mm	+ +	20.0	Moderately sensitive
	Chloramphenicol	9mm	+ + +	60.0	Quite sensitive
	Sulphamethoxazole trimethoprim	11mm	+ + + +	73.3	Highly sensitive
Escherichia coli	Tetracycline	Omm	-	0.0	Resistant
	Ampicillin	Omm	-	0.0	Resistant
	Gentamicin	8mm	+ + +	53.3	Quite sensitive
	Kanamycin	7mm	+ + +	46.6	Quite sensitive
	Neomycin	5mm	+ +	33.3	Moderately sensitive
	Baquiloprim Sulphadimidine	4.5mm	+ +	30.0	Moderately sensitive
	Chloramphenicol	8mm	+ + +	53.3	Quite sensitive
	Sulphamethoxazole trimethoprim	Omm	-	0.0	Resistant sensitive
Proteus vulgaris	Tetracycline	Omm	-	0.0	Resistant
	Ampicillin	Omm	-	0.0	Resistant
	Gentamicin	Omm	-	0.0	Resistant
	Kanamycin	9mm	+ + +	60.00	Quite sensitive
	Neomycin	6mm	+ +	40.00	Moderately sensitive
	Baquiloprim Sulphadimidine	Omm	-	0.0	Resistant
	Chloramphenicol	11mm	+ + + +	73.3	Highly sensitive
	Sulphamethoxazole trimethoprim	Omm	-	0.0	Resistant
Pseudomonas aeruginosa	Tetracycline	10mm	+ + +	66.6	Quite sensitive

	Ampicillin	11mm	+ + + +	73.3	Highly sensitive
	Gentamicin	4mm	+ +	28.6	Moderately sensitive
	Kanamycin	2mm	+	13.3	Weakly sensitive
	Neomycin	4mm	+ +	26.6	Moderately sensitive
	Baquiloprim	Omm		0.0	Resistant
	Sulphadimidine				
	Chloramphenicol	13mm	+ + + +	86.6	Highly sensitive
	Sulphamethoxazole trimethoprim	12mm	+ + + +	80.0	Highly sensitive
Micrococcus luteus	Tetracycline	11mm	+ + + +	73.3	Highly sensitive
	Ampicillin	10mm	+ + +	66.6	Quite sensitive
	Gentamicin	2mm	+	13.3	Weekly sensitive
	Kanamycin	4mm	+ +	26.6	Moderately sensitive
	Neomycin	3mm	+ +	20.0	Moderately sensitive
	Baquiloprim	8mm	+ + +	53.3	Quite sensitive
	Sulphadimidine				
	Chloramphenicol	14mm	+ + + +	93.3	Highly sensitive
	Sulphamethoxazole trimethoprim	13mm	+ + + +	86.6	Highly sensitive
Stomatococcus mucilaginosus	Tetracycline	10mm	+ + +	66.6	Quite sensitive
	Ampicillin	12mm	+ + + +	80.0	Highly sensitive
	Gentamicin	5mm	+ +	33.3	Moderately sensitive
	Kanamycin	4mm	+ +	26.6	Moderately sensitive
	Neomycin	3mm	+ +	20.0	Moderately sensitive
	Baquiloprim	11mm	+ + + +	73.3	Highly sensitive
	Sulphadimidine				
	Chloremphenicol	13mm	+ + + +	86.6	Highly sensitive
	Sulphamethoxazole trimethoprim	15mm	+ + + +	100.0	Highly sensitive

Absence of clear zone around discs	-
Clear zone with 1-2mm	+
Clear zone with 2-5mm diameter around discs	+ +
Clear zone with 5-10mm diameter around discs	+ + +
Clear zone with 10-15mm diameter around discs	+ + + +

- = resistant + = weakly sensitive + + moderately sensitive + + + = quite sensitive + + + + = highly sensitive

Method: Before conducting the sensitivity test, the surface of Muller Hinton agar was dried by incubating at 37°C for 30 minutes. The isolated colonies were selected and suspended in normal saline and then colour was matched with barium chloride to record the bacterial cell population. The sterile cotton swab was dipped in the bacterial suspension and then rolled over the surface of the agar medium and covered evenly with the bacterial suspension and placed in incubator for 30 minutes to get dried. Eight different discs were placed over the surface of agar plate. The culture was incubated for 24 hours and after that period results were recorded with the annotations and percentage of susceptibility calculated as described by Bauer *et al*. (1966) through the size of sensitivity zone around disc.

Results and Discussion

Eight different antibiotics were used to demonstrate the sensitivity of bacterial species identified from wound samples of various species of domestic animals. The results are shown in Table 1 and Fig. 1.

Streptococcus pyogenes organisms were found highly sensitive to sulphamethoxazole trimethoprim, tetracycline, and chloramphenicol, and their sensitivity recorded as 100, 80 and 73.3% respectively. While moderate effects of the other drugs to *Streptococcus pyogenes* were noted. The antibiotics were kanamycin, neomycin and gentamicin and their levels of sensitivity against organisms were 26.6, 20 and 13.1% respectively. Ampicillin was also found quite effective against above species, but no effect of baquiloprim sulphadimidine was seen during investigation. A similar trend of sensitivity of *Streptococcus uberis* for various antibiotics was observed. During the preSent investigation only ampicillin was highly effective against *Streptococcus uberis.* The effects of the other drugs are given in the same Table 1. and Fig. 1.

The tendency of sensitivity of the antibiotics against in genus *Staphylococcus* groups *(Staphylococcus aureus* and *Staphylococcus intermedius)* was somewhat different from genus *Streptococcus.* Sulphamethoxazole trimethoprim was recorded the most effective drug while ampicillin was second highly effective against *Staphylococcus* and showed averagely 90% sensitivity. The efficacy of sensitivity of the other antibiotics to *Staphylococcus* species is presented in Table 1 and Fig. 1.

During the study antibiotic sensitivity was also carried-out on *Corynebacterium diphtheriae* and *Corynebacterium pyogenes* and their results are presented in the same Table 1 and Fig. 1. Both the species were found highly sensitive to tetracycline and sulphamethoxazole trimethoprim and their sensitivity to above antibiotics were recorded as 73.3, 73.3, 80 and 73.3% respectively. The effects of the other drugs are also given in Table 1 and Fig.1.

The response of *Escherichia coli* to antibiotics was quite different from other bacterial species. All the bacterial species described before were highly sensitive to tetracycline, ampicillin and sulphamethoxazole trimethoprim while *Escherichia coli* was found highly resistant to above drugs and sensitivity was recorded as 0%. During this *in vitro* antibiotic sensitivity investigation, *Escherichia coil* was observed quite sensitive to gentamicin and chloramphenicol and their intensity was recorded as 53.3% respectively. The effects of the other drugs are demonstrated in the same Table 1 and Fig. 1. *Proteus vulgaris* was found highly sensitive to chloramphenicol this drug showed 73.3% effect against *Proteus vulgaris* and quite and moderately sensitive to kanamycin and neomycin respectively and showed 60 and 40% sensitivity. While the species found resistant to other drugs is given in Table 1 and Fig. 1. It is clear from the present investigation that every species of bacteria has it's own nature of drug sensitivity and this whole may be due to chemical composition and also anatomical structure of the species where these drugs inhibit or alter the physicochemical nature of the cell. The results about drug sensitivity of *Pseudomonas aeruginosa* species are presented in the same Table 1 and Fig. 1. During this survey, baquloprim sulphadimidine showed no any effect against the above species. However, other antibiotics showed varying degree of sensitivity against the bacteria identified. The most effective drugs noted were chloramphenicol, sulphamethoxazole trimethoprim, ampicillin and tetracycline and showed 86.6, 80, 73.3 and 66.6% effect respectively

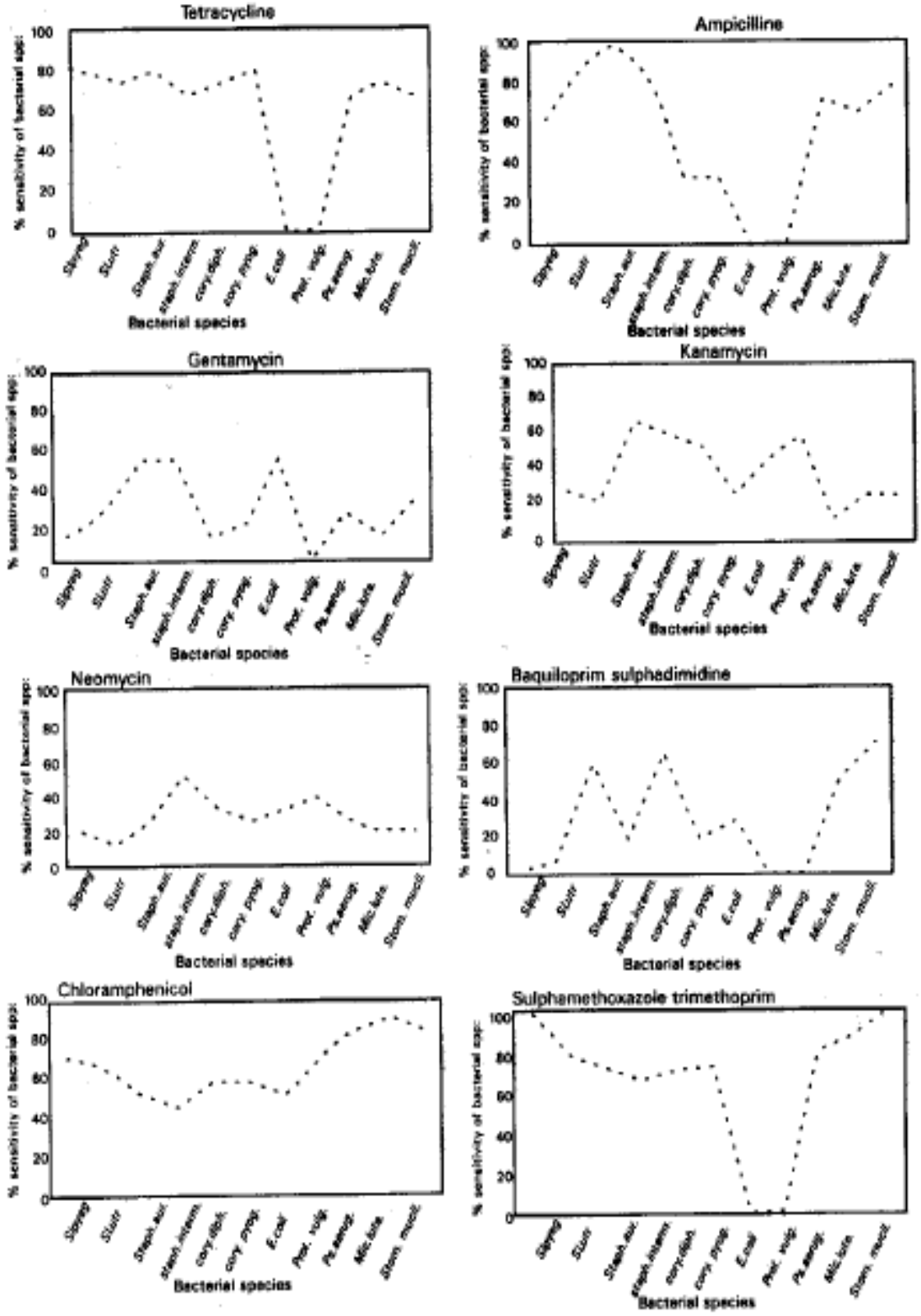

Fig. 1: The percentage of sensitivity of each bacterial species to various antibiotics observed during the present survey

against *Pseudomonas aeruginosa.* During current investigation on sensitivity of bacterial organisms, only these two groups *Micrococcus luteus* and *Stomatococcus mucilaginosus* were observed as the bacteria which showed sensitive to most of the antibiotics used. The results regarding drug sensitivity of the above two organisms are given in Table 1 and Fig. 1. The highly effective drugs against these organisms recorded were sulphamethoxazole trimethoprim, chloramphenicol, tetracycline and ampicillin. The moderate antibiotics against *Micrococcus luteus* and *Stomatococcus mucilaginosus* observed were gentamicin, kanamycin, neomycin and baquiloprim sulphadimidine. It is concluded from the present survey that both the organisms were similar in their nature and showed sensitivity to all drugs used. But a little variation in the sensitivity of the organisms to all antibiotics were recorded during the present study. The findings regarding antibiotic susceptibility of *Streptococcus pyagenes* and *Streptococcus uberis* recognized from wound samples of domestic animals to ampicillin, kanamycin, gentamicin and tetracycline similar to the results of Mathews *et al*. (1992) who recorded the susceptibility of the above species to ampicillin, kanamycin, gentamicin and tetracycline. During the present survey different antibiotics were applied to demonstrate the sensitivity of *Staphylococcus aureus* and *Staphylococcus intermedius.* Both the species were found highly sensitive to sulphamethoxazole trimethoprim and ampicillin and recorded as 90% against the above species. Awad-Masalmeh *et al*. (1988) did not carry sensitivity of *Staphylococcus aureus* and *Staphylococcus intermedius* by the above drugs.

The drugs used and susceptibility tested in the present study against *Staphylococcus aureus* and *Staphylococcus intermedius* by gentamicin, neomycin, chloramphenicdl, tetracycline and ampicillin and their percentage as 53.3, 26.6, 53.3, 80 and 100 and 53.3, 53.3, 46.6, 66.6 and 80 were not in accordance to Awad-Masalmeh *et al*. (1988) who tested gentamicin, neomycin, tetracycline, chloramphenicol and ampicillin and recorded susceptibility to the above species as 4.8, 11, 28, 39.5, and 16.9% respectively. Whereas the effects of ampicillin, chloramphenicol, neomycin and tetracycline and the susceptibility of *Staphylococcus aureus* was 23, 70, 91, 74 and 20% as recorded by Ayhan and Aydin (1991) are in line of the present investigation. A similar results regarding the susceptibility of the above species to various antibiotics as demonstrated in the present study were also reported by Zurzul and Katic (1991), Caracappa *et al.* (1991) and Mathews *et al.* (1992). Mathews *et al.* (1992) who found 65 to 67% sensitivity to *Staphylococcus aureus* by tetracycline and ampicillin respectively. In the present study *Corynebacterium diphtheriae* and *Corynebacterium pyogenes* were observed highly sensitive to sulphamethoxazole trimethoprim and tetracycline but unfortunately we did not use penicillin in this survey (Table 1 and Fig. 1). In the scientific literature only two references were found regarding the sensitivity of *Corynebacterium diphtheria* to penicillin and noted highly active but did not mention the percentage of susceptibility (Lee *et al*., 1944). Therefore, it was very difficult to compare present findings to the results of other workers.

Escherichia coli was observed quite sensitive to gentamicin and chloramphenicol and susceptibility was recorded as 53.3% (Table 1 and Fig. 1) while ineffective drugs noted were sulphameth-oxazole trimethoprim and tetracycline, that showed 0% action against the above species. Singh *et al*. (1992) assessed the susceptibility of *Escherichia coli* by 14 different antibiotics through the disc diffusion technique and found tetracycline most ineffective against *Escherichia coli.* Therefore the results of the present investigation do agree with the findings of the above author. Present findings also partially agree with the results of De Aguayo *et al.* (1992) and Riera-i-Pugadas (1992) who found *Escherichia coli* resistant to tetracycline but not to other drugs that we had used and found *Escherichia coli* susceptibility. Malik (1963) also noted resistant to tetracycline.

Proteus vulgaris organisms were detected highly sensitive to chloramphenicol but less sensitive to kanamycin and neomycin and other drugs were resistant. Dinev *et al*. (1987) carried-out an antibiotic sensitivity against *Proteus vulgaris* and gentamicin was found highly effective. The other antibiotics observed unlikely successful were chloramphenicol, tetracycline and streptomycin.

The results of the present survey on the susceptibly to antibiotics are not in complete agreement to the findings of Dinev *et al*. (1987) because it depends upon the environmental conditions provided to the species and also potency of drugs used. The most active antibiotics recorded against wound organism *Pseudomonas aeruginosa* during this study were chloramphenicol, sulphamethoxazole trimethoprim, ampicillin and tetracycline and its susceptibly was recorded as 86.6, 80, 73.3 and 66.6% respectively were also demonstrated by Dinev *et al*. (1987) who found gentamicin as a successful drug against *Pseudomonas aeruginosa* while unlikely successful antibiotics were chloramphenicol, tetracycline and streptomycin.

Therefore the findings of this investigation are not in complete agreement because we found highly effective and they mentioned unlikely successful drugs against *Pseudomonas aeruginosa.* This controversy in results may be due to drug efficacy and conditions provided to the species *during* experimental work. Unfortunately we could not find any information regarding antibiotic sensitivity to *Micrococcus luteus* and *Stomatococcus mucilaginosus* from available literature. Therefore, it was very difficult to compare the present results with the findings of other workers on these micro-organisms.

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