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Full Length Research Paper

Antimicrobial susceptibility of bacterial species identified from mastitic milk samples of camel

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Twelve different antibiotics were used against bacterial species to record their sensitivity. The antibiotics were amikacin, amoxicillin, ampicillin, cephalexin, chloramphenicol, erythromycin, gentamycin, kanamycin, neomycin, ofloxacin, sulphamethoxazole trimethoprim and tetracycline. The species that showed sensitivity to amikacin were: *Corynebacterium pyogenes* (100%), *Bacillus cereus* (91.6%), *Staphylococcus aureus* (85.7%) and *Pseudomonas aeruginosa* (66.6%). Whereas, *B. cereus* (100%), *C. pyogenes* (70.5%), *Micrococcus luteus* (78.5%), *Pasteurella haemolytica* (100%), *Pasteurella multocida* (100%), *P. aeruginosa* (72.2%) and *S. aureus* (100%) were observed to be highly sensitive to tetracycline. The species *S. aureus* (100%), *P. aeruginosa* (100%) and *B. cereus* (91.6%) were highly sensitive to sulphamethoxazole trimethoprim. The species *Escherichia coli* (100%), *M. luteus* (100%), *P. haemolytica* (92.8%) and *P. multocida* (93%) showed sensitivity to chloramphenicol. Moreover, *P. haemolytica* (100%), *P. multocida* (100%), *C. pyogenes* (70.5%) and *S. aureus* (85.7%) were sensitive to neomycin. The other species recorded as highly sensitive to cephalexin were: *C. pyogenes* (100%), *P. multocida* (80%), *P. aeruginosa* (72.2%) and *S. aureus* (85.7%), respectively.

Key words: Antimicrobial susceptibility, bacterial organisms, mastitic milk.

INTRODUCTION

Mastitis in bovine has become extremely complex and the costliest disease in Indo Pakistan. It affects 50% of herd population (Garg, 2001). It has been estimated that mastitis alone can cause approximately 70% of all available losses incurred during milk production. One important reason for treatment failure is assumed to be indiscriminate use of antibacterials without testing *in vitro* sensitivity of causal organisms (Saxena et al., 1993).

The practice in one hand increases economic losses and on the other hand, results in development of resistance to commonly used antimicrobials (Owens et al., 1997). The antimicrobial susceptibility of bacterial species to various drugs studied and recorded all over the world and many recommendations have been made by different workers on the susceptibility of organisms to various antibiotics. Barbour et al. (1985) used the Muller Hinton agar for the susceptibility testing of 118 isolates by the Disc Diffusion Method adopted by Bauer et al. (1966). For fastidious organisms, the Muller Hinton agar was supplemented with 5% sheep blood. Most frequent bacterial flora in 205 milk samples from different camels recorded were: *Staphylococcus aureus, Micrococcus, Streptococcus* and *Corynebacterium* species. Different bacterial isolates from camels' milk differ in their susceptibility patterns to six antimicrobial agents. While testing *in vitro* susceptibility, 118 bacterial isolates showed their sensitivity to drugs in decreasing order of ampicillin (10 µg), chloramphenicol (30 µg), gentamycin (10 µg), penicillin (10 units), streptomycin (10 µg) and tetracycline (30 µg). The *in-vitro* susceptibility testing of the bacterial isolates indicated that chloramphenicol, gentamycin and ampicillin were the most effective drugs. However, other bacterial flora showed the greatest resistance to penicillin, streptomycin and tetracycline. Rind and Shaikh (2001) reported that *Corynebacterium pyogenes* showed its sensitivity to gentamycin, kanamycin, chloramphenicol, sulphamethoxazole trime-thoprim and tetracycline while resistant to polymyxin B. Rind and

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Khan (2000) also observed that the *C. pyogenes* was highly sensitive to sulphamethoxazole trimethoprim and tetracycline and its sensitivity against the drugs was recorded as 80 and 73.3%, respectively. Keeping in view the susceptibility and resistance of bacterial species to antibiotics, the present study was designed to demonstrate the sensitivity of the organisms to antibiotics that causes mastitis in camel.

MATERIALS AND METHODS

Seventy-eight clinical mastitic milk samples from different herds of camels were collected in sterilized bijoux bottles (completely wrapped/covered with aluminum foil) and brought to the laboratory of the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam and Central Veterinary Diagnostic Laboratory, Tando Jam. Before collection of samples, the tips of mastitic teats were cleaned with cotton wool moistened with 70% alcohol and few strips of milk were discarded to avoid contamination as much as possible. All glassware, new and used were kept in 1% HCl solution overnight. The glassware was removed from solution and washed well with distilled water several times, then dried in oven at 65°C for one and half hour (Gabbar, 1992). The media was prepared and incubated by mastitic milk samples for the identification of bacterial species which was described by Rind and Khan (2000). Both solid and liquid media were used. The solid comprises nutrient, blood and MacConkey's agar, while the broth consists of nutrient broth which 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 Gabbar (1992). A few biochemical tests were also carried-out to confirm the specific chemical characteristics of the organism. For this purpose, oxidase, catalase, coagulase, indole, Voges Proskauer, urease, methyl red, gelatin liquefaction, Simon's citrate, H_2S production and TSI tests were conducted (Gabbar, 1992) for sugar fermentation of each species as tool for their identification; nine different sugars of 1% were prepared and used for each isolates bacterium as prescribed by Gabbar (1992). The sugars used were: Mannose, xylose, inositol, galactose, mannitol, glucose, maltose, creatinin and dulcitol. For the sensitivity of the organisms to different antibiotics, the discs used were amikacin, amoxicillin, ampicillin, cephalexin, chloramphenicol, erythromycin, gentamycin, kanamycin, neomycin, ofloxacin, sulphamethoxazsole trimethoprim and tetracycline. All discs were of 30 mg. Testing the antibiotics sensitivity of the organism was by Bauer et al. (1966) method, the following materials were brought and used: Mueller-Hinton agar plates, 150 x 15 mm, 4 to 6 mm deep medium, sterile saline, barium chloride standard, sterile cotton wool or sterile swabs, sterile forceps, ruler and sensitivity chart.

Minimal inhibitory concentration (MIC) values of bacterial organism were analyzed for thirteen different antimicrobials namely, amikacin, amoxicillin, ampicillin, cephalexin, chloramphenicol, erythromycin, gentamycin, kanamycin, neomycin, ofloxacin, sulphamethoxazole trimethoprim and tetracycline. The disc diffusion method as described by Miles (1996) was employed and the interpretation was made as per the zone size interpretation chart provided by the manufacturer of the disc. Before conducting the sensitivity test, the surface of Muller Hinton agar was dried by incubating at 37°C for 30 min. 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 min to get dried. The culture was incubated for 24 h and after that period, results were recorded with the annotation and percentage of susceptibility calculation as described by Bauer et al. (1966) through the size of sensitivity zone around disc.

RESULTS AND DISCUSSION

Twelve different antibiotics were used against bacterial species identified from mastitic milk samples of camel. The results are shown in Table 1. *Bacillus cereus* was recorded as highly sensitive to ampicillin, tetracycline and their action against the species were observed as 100%. While, sulphamethoxazole/trimethoprim and amikacin were found to be second most effective drugs (91.6%) followed by gentamycin, cephalexin, ofloxacin and their sensitivity against the species was recorded as 83.3%. Moderate effects of other antibiotics against *B. cereus* were also noted. The antibiotics kanamycin and neomycin were observed as 75% effective, while chloramphenicol is 58.3%. The other antibiotics including amoxicillin, erythromycin and their level of sensitivity against the species were also recorded during investigation (Table 1). Similar trend of susceptibility was observed by Mishra et al. (1996) who investigated *in vitro* sensitivity of *B. cereus* and other bacterial isolates recovered from goat mastitic milk samples. Antibiotic sensitivity indicated that, these microorganisms were sensitive to gentamycin (100%), tetracycline (55%), erythromycin and ampicillin (25%), whereas, all the isolates were resistant to penicillin. Dewani (2000) also observed that *B. cereus* was a resistant species to penicillin and sulphamethoxazsole/ trimethoprim, but showed susceptibility to gentamycin, erythromycin, clindomycin and chloramphenicol.

The tendency of efficacy of the antibiotics against *C. pyogenes* was also tested *in-vitro*. Amikacin and cephalexin were found to be highly active drugs against *C. pyogenes* and their efficacy was recorded as 100%, while efficacy of ofloxacin against the species was noted as 88.2%. Other antibiotics used include gentamycin, neomycin, tetracycline and sulphamethoxazsole/ trimethoprim and their efficacy was observed as 70.5%. Moderate effects of amoxicillin, kanamycin and chloramphenicol against the bacterium were also observed during the study (Table 1) and ofloxacin, both showed 100 and 88.2% activity against the species. Almost similar results were also recorded by Rind and Shaikh (2001). Rind and Khan (2000) observed that *C. pyogenes* was highly sensitive to gentamycin (93.3%), kanamycin (90%), chloramphenicol (86.6%), tetracycline (80%) and sulphamethoxazole (73.3%).

During the present investigation, *Escherichia coli* was observed as highly sensitive to chloramphenicol and the susceptibility was observed as 100%, followed by ofloxacin (84.6%), ampicillin (76.9%), amoxicillin (69.2%) and amikacin (69.2%), and quite susceptible to kanamycin

Table 1. Antimicrobial drug susceptibility of bacterial species.

Bacterial species	Antibiotic discs Used	Zone around discs	Indication of sensitivity	Sensitivity percentage	Degree of sensitivity
Bacillus cereus	Amikacin	11mm	++++	91.6	Highly sensitive
	Amoxicillin	5mm	++	41.6	Moderately sensitive
	Ampicillin	12mm	++++	100	Highly sensitive
	Cephalexin	10mm	+++	83.3	Quit sensitive
	Chloramphenicol	7mm	++	58.3	Moderately sensitive
	Erythromycin	4mm	++	33.3	Moderately sensitive
	Gentamycin	10mm	+++	83.3	Quit sensitive
	Kanamycin	9mm	+++	75	Quit sensitive
	Neomycin	9mm	+++	75	Quit sensitive
	Ofloxacin	10mm	+++	83.3	Quit sensitive
	Sulphamethoxazsole	11mm	++++	91.6	Highly sensitive
	Tetracycline	12mm	++++	100	Highly sensitive
Corynebacterium pyogenes	Amikacin	17mm	++++	100	Highly sensitive
	Amoxicillin	10mm	+++	58	Quit sensitive
	Ampicillin	0mm	-	0	Resistant
	Cephalexin	17mm	++++	100	Highly sensitive
	Chloramphenicol	3mm	+	7.6	Sensitive
	Erythromycin	0mm	-	0	Resistant
	Gentamycin	12mm	++++	70.5	Highly sensitive
	Kanamycin	10mm	+++	58	Quit sensitive
	Neomycin	12mm	++++	70.5	Highly sensitive
	Ofloxacin	15mm	++++	88.2	Highly sensitive
	Sulphamethoxazsole	12mm	++++	70.5	Highly sensitive
	Tetracycline	12mm	++++	70.5	Highly sensitive
Escherichia coli	Amikacin	9mm	+++	69.2	Quit sensitive
	Amoxicillin	9mm	+++	69.2	Quit sensitive
	Ampicillin	10mm	+++	76.9	Quit sensitive
	Cephalexin	5mm	++	61.5	Moderately sensitive
	Chloramphenicol	13mm	++++	100	Highly sensitive
	Erythromycin	6mm	++	46.1	Moderately sensitive
	Gentamycin	7mm	++	53.8	Moderately sensitive
	Kanamycin	8mm	+++	61.5	Quit sensitive
	Neomycin	5mm	++	38.4	Moderately sensitive
	Ofloxacin	11mm	+++	84.6	Quit sensitive
	Sulphamethoxazsole	0mm	-	0	Resistant
	Tetracycline	0mm	-	0	Resistant
Micrococcus luteus	Amikacin	8mm	+++	53.3	Quit sensitive
	Amoxicillin	9mm	+++	60	Quit sensitive
	Ampicillin	10mm	+++	71.4	Quit sensitive
	Cephalexin	11mm	+++	78.5	Quit sensitive
	Chloramphenicol	14mm	++++	100	Highly sensitive
	Erythromycin	7mm	++	46.6	Moderately sensitive
	Gentamycin	2mm	+	13.3	Sensitive
	Kanamycin	4mm	++	26.6	Moderately sensitive
	Neomycin	3mm	+	21.4	Sensitive
	Ofloxacin	10mm	+++	71.4	Quit sensitive
	Sulphamethoxazsole	13mm	+++	86.6	Highly sensitive
	Tetracycline	11mm	+++	78.5	Highly sensitive

Table 1. Contd.

Pasteurella haemolytica	Amikacin	9mm	++	64	Quit sensitive
	Amoxicillin	8mm	++	57	Quit sensitive
	Ampicillin	9mm	++	64	Quit sensitive
	Cephalexin	11mm	+++	78.5	Quit sensitive
	Chloramphenicol	13mm	++++	92.8	Highly sensitive
	Erythromycin	7mm	++	50	Moderately sensitive
	Gentamycin	10mm	+++	71.4	Quit sensitive
	Kanamycin	10mm	+++	71.4	Quit sensitive
	Neomycin	14mm	++++	100	Highly sensitive
	Ofloxacin	12mm	++++	85.7	Highly sensitive
	Sulphamethoxazsole	8mm	+++	57.1	Quit sensitive
	Tetracycline	14mm	++++	100	Highly sensitive
Pasteurella multocida	Amikacin	10mm	+++	66.6	Quit sensitive
	Amoxicillin	7mm	+	46.6	Moderately sensitive
	Ampicillin	8mm	++	53.3	Quit sensitive
	Cephalexin	12mm	++++	80	Highly sensitive
	Chloramphenicol	14mm	++++	93	Highly sensitive
	Erythromycin	9mm	+++	60	Quit sensitive
	Gentamycin	10mm	+++	66.6	Quit sensitive
	Kanamycin	10mm	+++	66.6	Quit sensitive
	Neomycin	15mm	++++	100	Highly sensitive
	Ofloxacin	11mm	+++	73.3	Quit sensitive
	Sulphamethoxazsole	8mm	+++	53.3	Quit sensitive
	Tetracycline	15mm	++++	100	Highly sensitive
Pseudomonas aeruginosa	Amikacin	12mm	++++	66.6	Highly sensitive
	Amoxicillin	9mm	+++	50	Quit sensitive
	Ampicillin	11mm	+++	61.1	Quit sensitive
	Cephalexin	13mm	++++	72.2	Highly sensitive
	Chloramphenicol	8mm	+++	44.4	Quit sensitive
	Erythromycin	11mm	+++	61.1	Quit sensitive
	Gentamycin	12mm	++++	66.6	Highly sensitive
	Kanamycin	10mm	+++	55.5	Quit sensitive
	Neomycin	11mm	+++	61.1	Quit sensitive
	Ofloxacin	14mm	++++	77.7	Highly sensitive
	Sulphamethoxazsole	18mm	++++	100	Highly sensitive
	Tetracycline	13mm	++++	72.2	Highly sensitive
Staphylococcus aureus	Amikacin	12mm	++++	85.7	Highly sensitive
	Amoxicillin	9mm	+++	64.2	Quit sensitive
	Ampicillin	10mm	+++	71.4	Quit sensitive
	Cephalexin	12mm	++++	85.7	Highly sensitive
	Chloramphenicol	8mm	+++	57	Quit sensitive
	Erythromycin	6mm	++	42.8	Moderately sensitive
	Gentamycin	11mm	+++	78.5	Quit sensitive
	Kanamycin	12mm	++++	85.7	Highly sensitive
	Neomycin	12mm	++++	85.7	Highly sensitive
	Ofloxacin	12mm	++++	85.7	Highly sensitive
	Sulphamethoxazsole	14mm	++++	100	Highly sensitive
	Tetracycline	14mm	++++	100	Highly sensitive

- = Resistance; + = weakly sensitive; ++ = moderately sensitive; +++ = quite sensitive; ++++ = highly sensitive.

(61.5%), cephalexin (61.5%), gentamycin (53.8%), erythromycin (46.1%) and neomycin (38.4%). The drugs observed to be ineffective against the species were tetracycline and sulphamethoxazsole (Table 1). Similarly, *E. coli* showed high sensitivity to chloramphenicol and its susceptibility was observed as 100%, while it showed moderate sensitivity to ofloxacin (84.6%), ampicillin (76.9%), amoxicillin (69.2%) and amikacin (69.2%). Rind and Khan (2000) assessed the susceptibility of *E. coli* by 10 different antibiotics through disc diffusion technique and found the species to be less susceptible to gentamycin and chloramphenicol and was recorded to be 53.3%, while ineffective drugs against the species were sulphamethoxazole trimethoprim and tetracycline, both showed 0% action against the above species.

Micrococcus luteus was found to be 100% sensitive to chloramphenicol, 86.6% to sulphamethoxazole, 78.5% to tetracycline, 71.4% to ampicillin, 78.5% to cephalexin and 71.4% to ofloxacin. The tendency of efficacy of other drugs against the species is also measured and recorded in the same Table 1. Rind and Khan (2000) showed that highly effective drugs against the organism were sulphamethoxazole/trimethoprim, chloramphenicol, tetracycline and ampicillin. The findings regarding antibiotic susceptibility of *Pasteurella haemolytica* recog-nized from camel mastitic milk samples to tetracycline and neomycin was recorded as 100%. The susceptibility of the species to chloramphenicol (92.8%), ofloxacin (85.7%) and cephalexin (78.5%) was also noted, while it showed moderate susceptibility to gentamycin and kanamycin and it was observed as 71.4%, and followed by ampicillin and amikacin, with activity against the species observed as 64%.

The susceptibility of the organism to erythromycin, amoxicillin and sulphame-thoxazole was observed and presented in Table 1. Dewani (2000) also investigated susceptibility of *P. haemolytica* to different antibiotics. The organism was found to be highly sensitive to tetracycline and neomycin and was recorded as 100%.

Pasteurella multocida was recorded as 100% sensitive to neomycin and tetracycline. Other antibiotics including chloramphenicol, cephalexin and ofloxacin were also observed to be highly effective against the species and their efficacy was recorded as 93, 80 and 73.3%, respectively. Gentamycin, kanamycin and amikacin were found to be moderately active against the organism and their efficacy was measured as 66.6%. The efficacy of erythromycin, amoxicillin, ampicillin and sulphamethoxazsole was also demonstrated against the species and presented in Table 1. Rind and Shaikh (2001) reported that organism *P. multocida* was highly sensitive to neomycin and tetracycline (100%), followed by chloramphenicol and tetracycline and their effects on the species were recorded as 93.3 and 96.6%, respectively.

The results on drug sensitivity of *Pseudomonas aeruginosa* are presented in Table 1. During the present study, the organism was observed as 100% sensitive to sulphamethoxazsole. Whereas, ofloxacin, cephalexin, tetracycline, gentamycin, amikacin, neomycin, ampicillin and erythromycin were seen as moderately active against *P. aeruginosa* and its susceptibility to above drugs was 77.7, 72.2, 72.2, 66.6, 66.6, 61.1, 61.1 and 61.1%, respectively. The other antibiotics, chloramphenicol, amoxicillin and kanamycin were also tested against the species and their results are presented in Table 1. Rind and Shaikh (2001) also showed that gentamycin, kanamycin, chloramphenicol and sulphamethoxazole were highly effective against *P. aeruginosa* and their efficacy was recorded as 86.6, 80, 86.6 and 80%, respectively

The species, *S. aureus* was found to be highly sensitive to tetracycline and sulphamethoxazsole and their efficacy against the species was recorded as 100%, followed by neomycin, kanamycin, amikacin and cephalexin and ofloxacin which was recorded as highly effective against *S. aureus* (85.7%). The efficacy of the other antibiotics against *S. aureus* is also presented in Table 1. The effect of tetracycline, ampicillin, chloramphenicol and neomycin against *S. aureus* was recorded as 23, 70, 91, 74 and 20%, respectively (Ayhan and Aydin, 1991). A similar result regarding the susceptibility of the above species to various antibiotics as demonstrated in the present study were also reported by Methews et al. (1992) who observed sensitivity of *S. aureus* to tetracycline and ampicillin as 67%. Rind and Shaikh (2001) observed antibiogram sensitivity of *S. aureus* to tetracycline, gentamycin, chloramphenicol, kanamycin and sulphamethoxazole, and their sensitivity was noted as 80, 86.6, 86.6, 73.3 and 73.3%, respectively.

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