Research Article

Antibiogram of the *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus intermedius* isolated from the bovine frozen semen

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Abstract

The presence of harmful microbes may negatively influence semen quality. Antibiotic used in the semen extender should be evaluated in order to prevent contamination and infertility. In this study, Antibiogram evaluation of *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus intermedius* were detected from frozen semen of cattle. Different antibiotics such as amikacin, ampicillin, amoxicillin, cephalexin, erythromycin, gentamicin, neomycin, ofloxacin and sulphamethoxazole/trimethoprim were tested against the isolated bacterial species. Ofloxacin, amikacin, cephalexin and amoxicillin were highly effective against *Micrococcus luteus*. The organism was found equally effective to erythromycin, gentamycin and neomycin. Amikacin, ofloxacin, erythromycin, neomycin and ampicillin were the most effective against the *Pseudomonas aeruginosa*. However, amoxicillin, cephalexin and

sulphamethoxazole/trimethoprim were not shown any response. *Staphylococcus epidermidis* was found highly sensitive to amikacin. While antibiotics amoxicillin, erythromycin, and cephalexin completely failed to give response against the organism. *Staphylococcus intermedius* was noted highly sensitive to neomycin, amikacin and ampicillin. Overall, amikacin and neomycin were found the most effective antibiotics against these bacterial isolates determined from bovine semen samples.

Key words: Antibioqram; Bacterial species; Bovine; Semen

Introduction

Artificial insemination (AI) has been useful method that is practiced for breeding of cattle and other domestic animal species all over the world. This method is the best tool which is benefiting farmers to obtain high quality genetic potential from proven animals [1-2]. Routinely, semen packaged in straws approximately 0.25 ml or 0.5 ml in volume for thawing and storage. The thaw straws and polythene bags containing semen are transported using liquid nitrogen [3, 4]. However, there is a risk of contamination of semen from microbes during the packaging, storage and transportation can influence quality of semen and reproductive efficiency [5].

Generally, fresh semen contains some of non-pathogenic microbes that are neither harmful nor affecting the semen quality. However, the presence of large number or harmful microbes may negatively influence semen quality [6]. Frozen semen could get contaminated with pathogenic and non-pathogenic microbial agents during processing and handling. The microbes in the semen can transfer serious diseases in recipient animals and/or could be potential source of infections in different parts of genital tract [7, 8].

The semen production centers have to ensure that the processed semen should be free from environmental microbes and donor bulls. The presence of *Acinetobacter cacloaceticus*, *Brucella suis*, *Chlamydophila abortus*, *Coxiella burnetti*, *Enterobacter-coccus*, *Enterobacter cloacae*, *Escherichia coli*, *Histophilus somni*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pantoeau*

agglomerans, *Staphylococcus sciuri*, *Ureaplasma diversum*, *Stenotrophomonas maltophilia*, *Micrococcus*, *Leptospira*, *Corynebacteriu* and *Flavobacterium* species been reported in the frozen semen of farm animals [9-17]. Furthermore, the presence of bacterial species in bull semen may varies according to different breeds [18].

In normal practice, antibiotics are added to semen extender in order to control various microorganisms and to improve the quality of bovine semen [19]. Generally, combination of streptomycin and penicillin are added to diluents in extender for bovine semen [20, 21]. The combination of lincospectin, gentamycin and tylosin diluted in bovine semen extender that is sufficiently effective for controlling in microorganism such as; *Campylobacter fetus* *Mycoplasma* and *Pseudomonas* species [22]. In contrast, the combination of lincomycin, gentamicin, spectinomycin and tylosin (LGST) were added in bull semen extender to check the efficiency of this combination of the antibiotics against mycoplasma species. The combination failed to control growth of mycoplasma species in the semen extender contained LGST antibiotic mixture. This indicated the various antibiotics used in semen extender are not necessary to control infection or microbial contamination [23]. Traditionally, addition of pencillin in semen extender is not responding against different microbes e.g. brucella, cornyacteria, mycobacteria, vibrio, hemophillus, ureaplasmas and mycoplasmas species [24]. Antibiotic resistance is an emerging problem for different microorganism contaminants in frozen semen of cattle, used for artificial

insemination. Therefore, present study is designed to determine the efficacy of different antibiotics against the bacterial isolates identified from bovine semen used for artificial insemination.

Materials and methods

Bovine semen samples (one hundred) were obtained under sterile hygienic condition from the local semen production centers at Karachi and Rohri. The semen samples were contained in straws and sterilized bijou bottles in artificial insemination kits, which contained liquid nitrogen and brought to the Central Veterinary Diagnostic Laboratory, TandoJam, Pakistan.

Different dehydrated media were used for the culture or presence of any bacteria in the frozen semen samples. Dehydrated nutrient agar (Difco, 2000), MacConkey agar (Difco, 2000) and blood agar (Difco, 2000) were rehydrated according to recommendation of manufacturer. The medium was thoroughly mixed and autoclaved at 121°C, 15 lb pressure for 15 min. The blood agar medium was kept at room temperature and added with 5% sheep blood (defibrinated). These media were incubated aerobically at 37°C for 24 h and checked for microbial growth. The bovine semen samples were inoculated by streaking method on blood, nutrient and MacConkey's agar media. The bacterial colonies were observed for bacterial growth. The bacteria that were grown on the media, sub-cultured to obtain pure culture of isolated organism. The single colony was obtained from the sub-culture and analyzed for staining. The bacteria grown on different media were observed for morphological and colony characteristic.

The bacterial colonies were taken for the pure culture and for the biochemical characteristics and sugar fermentation abilities. Various biochemical tests e.g. oxidase, coagulase catalase, urease, aesculin test, gelatin liquefaction, bile tolerance, indole production, methyl blue, Hugh and

Leifson's test, methyl red, nitrate reduction Vogus-Proskauer test, triple sugar iron agar, Simmon's citrate and sugar fermentation tests were performed as prescribed by [25-27]. These biochemical and sugar fermentation tests were performed for the identification and confirmation of the isolates contained in the frozen semen samples.

The antibiotic sensitivity was performed as described by Bauer *et al.* [28]. The different antibiotics; amikacin, ofloxacin, neomycin, chloromphenicol, cephalixin, amoxicillin, kanamycin, ampicillin, gentamicin, sulphamethoxazole/ trimethoprim, tetracycline and erythromycin were used during the study. The Muller Hinton agar (Difco) was used and incubated at 37°C for 15 minutes. Bulks of pure culture colonies were suspended evenly in 2-4 sterile normal saline solution in order to match barium chloride standard for antibiotic sensitivity. A sterile cotton swab was dipped into the suspended and culture was smeared on the surface of Muller Hinton agar in such a way that all agar surface was covered evenly with the bacterial suspension, then incubated at 37°C for 15 minutes for plate to dry. The desired antibiotic disc were kept on agar surface with disc dispenser and lightly pressed with sterile forceps to make it adhere to surface. The plate was closed, wrapped in polythene bag, inverted (medium and disc upward) and incubated overnight at 37°C. The zone of inhibition was observed as a clear area, free from growth around the disc and a clear zone of inhibition made against organism. The zone of inhibition by the antibiotics was recorded in millimeter from the center of disc to the ending edges of zone. The antibiotic sensitivity was classified into highly, quite, moderately, weakly sensitive and resistant depending on the antibiotics, its contents in the disc and size of zone.

No clear zone around antibiotic discs -
 Clear zone with 1-2mm diameter around antibiotic discs +
 Clear zone with 2-5mm diameter around antibiotic discs ++
 Clear zone with 5-10mm diameter around antibiotic discs +++
 Clear zone with 10-15mm diameter around antibiotic discs ++++

Results and discussion

In this study, bovine semen samples were examined for the contamination or presence of bacterial species. Frozen bovine semen samples were found positive for *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus intermedius*. These bacterial organisms detected from the bovine semen samples were examined for the efficacy of different antibiotics.

The sensitivity of *Micrococcus luteus* was recorded high against the ofloxacin, amikacin, cephalixin and amoxicillin and their sensitivity against the species were observed 95.75%, 63.15, 60.50 and 60.50% respectively (Table 1). Previously, it has been reported that cephalixin, chloromphenicol, tetracycline, ampicillin were highly effective against the *Micrococcus luteus* [29, 30]. MICs of ofloxacin were elevated concentration 0.08 ug/ml effective to *Micrococcus species* [31]. The organism was found equally effective to erythromycin, gentamycin and neomycin the action against the organism recorded as 39.35%. The organism was weakly sensitive to sulphamethoxazole (4%). The minimum inhibition concentration (MIC) of nisin against the organism showed the concentration of 0.156 ug/ml [32].

Multidrug resistance is an emerging problem of *Pseudomonas aeruginosa* and getting

more attention due to nosocomial pathogen from the environment [33]. Antibiogram profile of *Pseudomonas aeruginosa* was investigated; amikacin, ofloxacin, erythromycin, neomycin and ampicillin were the most effective against the organism and their activity was noted 94%, 87.15%, 60.50%, 60.50% and 64.50% (Table 1) respectively. It has been reported that the organism showing high resistance to ceftazidime and ceftriaxone and comparatively less resistance to amikacin, ciprofloxacin, levofloxacin and ofloxacin. However, *Pseudomonas aeruginosa* showed lower resistance to combinations of cefoperazone, peperacillin, sulbactam and tazobactam [34]. Amikacin showed 73.52% activity against *Pseudomonas aeruginosa* [35]. In this study, amoxicillin, cephalixin and sluphamethoxazole were not affective against the *Pseudomonas aeruginosa*. Similarly, our findings are consentient to previous report [36] amoxicillin, moxifloxacin, ceftraxone, spiramycin, levofloxacin and cefotaxime were inactive against the micro-organisms. However, the micro-organism showed low sensitivity against the organism [35]. Decreasing the antibiotic susceptibility to *Staphylococcus* species is major concern in controlling its infections [37]. In this study, *Staphylococcus epidermidis* was found highly sensitive to amikacin 93.00% (Table 1). *Staphylococcus epidermidis* showed high sensitivity to amikacin, cephalotin, chloromphenicol, cephalolin, deoxycycline, norafloxacin and Vancomycin [38]. The organism was found quite sensitive to ampicillin, gentamycin, neomycin and ofloxacin its susceptibility against the antibiotics was recorded as 39.35%, 27.35%, 22.50% and 22.50% respectively (Table 1).

Table 1. Antibiotic sensitivity against the bacterial species isolated from bovine frozen semen

Bacterial species	Antibiotic disc	Inhibitory zone (mm)	Sensitivity (%)	Sensitivity Induction	Degree of sensitivity
<i>Micrococcus luteus</i>	Amoxycillin	12.55	60.50	++++	Highly sensitive
	Erythromycin	10.00	39.35	+++	Quite sensitive
	Gentamicin	10.00	39.35	+++	Quite sensitive
	Cephalexin	12.55	60.50	++++	Highly sensitive
	Amikacin	13.55	63.15	++++	Highly sensitive
	Ofloxacin	15.55	94.75	++++	Highly sensitive
	Neomycin	10.00	39.35	+++	Quite sensitive
	Sulphamethoxazole/Trimethoprim	01.00	04.00	+	Weakly sensitive
<i>Pseudomonas aeruginosa</i>	Neomycin	12.55	60.50	+++	Highly sensitive
	Erythromycin	12.55	60.50	++++	Highly sensitive
	Amikacin	15.00	94.00	++++	Highly sensitive
	Ofloxacin	14.50	87.15	++++	Highly sensitive
	Ampicillin	11.50	64.50	++++	Highly sensitive
	Amoxycillin	0	0	-	Not sensitive
	Cephalexin	0	0	-	Not sensitive
	Sulphamethoxazole/Trimethoprim	0	0	-	Not sensitive
<i>Staphylococcus epidermidis</i>	Neomycin	07.55	22.50	+++	Quite sensitive
	Gentamicin	08.55	27.32	+++	Quite sensitive
	Cephalexin	0	0	-	Not sensitive
	Ampicillin	10.00	39.35	+++	Quite sensitive
	Erythromycin	0	0	-	Not sensitive
	Amikacin	15.00	93.00	++++	Highly sensitive
	Amoxycillin	0	0	-	Not sensitive
	Ofloxacin	07.55	22.50	+++	Quite sensitive
<i>Staphylococcus intermedius</i>	Neomycin	14.00	83.48	++++	Highly sensitive
	Gentamicin	08.50	28.13	+++	Quite sensitive
	Cephalexin	0	0	-	Not sensitive
	Ampicillin	11.55	64.50	++++	Highly sensitive
	Erythromycin	0	0	-	Not sensitive
	Amikacin	12.55	60.50	++++	Highly sensitive
	Amoxycillin	0	0	-	Not sensitive
	Ofloxacin	06.55	19.46	+++	Quite sensitive

Previously reported data regarding *Staphylococcus epidermidis* support our findings [39]. In this study, antibiotics amoxicillin, erythromycin and cephalexin

completely failed to give response against the *Staphylococcus epidermidis*. It has been studied antibiotic pattern of *Staphylococcus epidermidis* and observed that high

antibiotic resistance against cefixime, pencillin, ceftazidime, oxacillin, cephepime and nalidixic acid [38]. The findings in this study are partially in agreement with previous study [40]. *In-vitro* susceptibility of *Staphylococcus epidermidis* and observed that their sensitivity was variable against the antibiotics benzyl penicillin, tetracycline, neomycin, chloromphenicol, erythromycin and gentamycin [40].

There is an increasing trend in antimicrobial resistance among clinical isolates of *Staphylococcus intermedius* of small animals [41]. The cells of *Staphylococcus intermedius* were noted highly sensitive to neomycin (83.48%), amikacin (60.50%) and ampicillin (64.50%) and quite sensitive against gentamycin (28.13%) and ofloxacin (19.46%). However, *Staphylococcus intermedius* showed resistance against cephalixin, amoxicillin and erythromycin. It was investigated that antibiotic resistance of *Staphylococcus intermedius* found below 10% against the amoxi-clavulanic acid and fluoroquinolones and cefalexin. also, the organism exhibited increasing resistance trend toward amoxicillin, ampicillin, enrofloxacin and cefovecin [41].

Conclusion

In summary, *Micrococcus luteus* was highly susceptible to ofloxacin, amikacin, cephalixin and amoxicillin and weakly responding to sulphamethoxazole. *Pseudomonas aeruginosa* was shown high activity to amikacin, ofloxacin, erythromycin, neomycin, ampicillin and conferring the resistance to amoxicillin, cephalixin and sulphamethoxazole. *Staphylococcus epidermidis* was found highly susceptible to amikacin and amoxicillin, while erythromycin and cephalixin were not effective against the organism. *Staphylococcus intermedius* was noted highly susceptible to neomycin, amikacin and ampicillin and weakly susceptible against gentamycin and

ofloxacin. The organism showed resistance against cephalixin, amoxicillin and erythromycin. Overall, amikacin and neomycin were found the effective antibiotics against these bacterial isolates determined from bovine semen samples.

Authors' contributions

Conceived and designed the study, SH Abro, R Abro, R Rind, AA Kamboh, AA Memon & AA Channa, conducted experiments: SH Abro, R Abro & H Baloch, Analysed data: SH Abro, H Wagan & B Wagan, Written manuscript: SH Abro, H Wagan & B Wagan.

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