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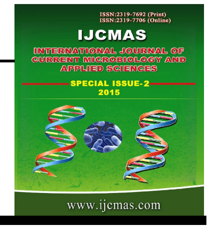

Original Research Article

Multi-drug Resistance of Micro-Organisms Isolated from Dog Skin and Saliva

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Keywords

Isolation of microorganisms, Dog skin, Dog saliva, Antibiotics resistance, Metal resistance, Multi-drug resistance

A B S T R A C T

Many micro-organisms are present on dog skin and in saliva. These organisms may acquire resistant to the antibiotics which are generally used, and the resistant pattern of these organisms is needed to study. Samples of skin and saliva of 10 dogs from a private dog house, were collected. Samples were processed and 25 different isolates (13 from skin samples i.e., Sk1-Sk13 and 12 from saliva samples i.e., Sv1-Sv12) were obtained. The morphological and biochemical characteristic identification was carried out. Out of 25 isolates, 18 isolates were Gram positive and 7 isolates (Sk11, Sv1, Sv2, Sv4, Sv5, Sv6, and Sv12) were Gram negative. All isolates were capsulated. Out of 25 isolates 23 were non-motile, whereas only 2 isolates (Sk10, Sv5) were motile. All isolates were observed to be catalase positive, whereas only 20 isolates were oxidase positive and 5 isolates (Sk7, Sk11, Sk12, Sv3, and Sv8) were oxidase negative. The resistance pattern of all these isolates for nine antibiotics and ten metals were studied by agar dilution method. Out of 25 isolates, only 7 isolates (Sk9, Sv4, Sv5, Sv7, Sv8, Sv10 and Sv12) showed resistance to Amoxicillin. Eleven isolates showed resistance for Tetracycline. All the isolates showed resistance for Metranidazole except one isolate (Sv8). Whereas for Neomycin, five isolates (Sk7, Sv1, Sv8, Sv10 and Sv11) were sensitive, and all other isolates showed resistance. Only 3 isolates showed resistance to Ampicillin, out of which 2 isolates Sv5 and Sv12 were Gram-negative and one isolate Sk3 was Gram-positive. Two Gram-negative, Sk11 and Sv12, and ten Gram-positive showed resistance to Ofloxacin. Six Gram-positive isolates showed resistance to this Ciprofloxacin antibiotic. All Gram-negative isolates inhibited at or below concentration of 512µg/ml. Cefadroxil was able to inhibit four Gram-positive and three Gram-negative organisms. Three Gram-positive and four Gram-negative organisms showed resistance to Gentamicin. All isolates showed resistance to all ten metals. Various resistance patterns were exhibited by different isolates for antibiotics and metals.

Introduction

Microbial flora has spatial and temporal complexity that usually differs by individual, body niche, age, geographic location, health status, diet and type of host (Salminen *et al.,* 1995; Savage, 1977). There is variation in the normal flora found in the oral cavity which depends on the area sampled (tooth enamel, tongue, gingivital surface, saliva) and the state of periodontal health (Lynne, 2000; Listgarten, 1976; Stjernquist-Destnik *et al.,* 1999). Colony counts of aerobic bacteria from moist areas such as the axilla or toe web spaces can reach 10^7 bacteria per cm^2, whereas dry areas such as the forearm or trunk may harbor 10^2 or fewer bacteria per cm^2 (Leyden *et al.,* 1987). Anaerobic bacteria are also present on human skin, with colony counts up to 10^6 bacteria per cm^2 (David, 2001). Nutrients like lipids and protein (keratin) are provided by skin for selected colonizing bacteria. This dry and slightly acidic environment may limit the types of microbes that can survive on normal skin. The organisms compete with each other for nutrients and space (David, 2001). Many external factors can alter the ecosystem of the skin, with resulting changes in microbial populations (Roth and James 1988). Numerous bacteria have been cultivated from normal skin (Leyden *et al.,* 1987; Roth and James, 1988). These include *Staphylococci*, *Micrococci*, *Corynebacteria*, *Brevibacteria*, *Propionibacteria*, and A*cinetobacter* species (David, 2001).

There is growing antibiotic resistance found among many strains of pathogens in animals. Some have become resistant to many antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance (Hiroshi Nikaido, 2009).

Ever since the discovery and subsequent clinical use of antibiotics, resistance to these agents has been observed (Wright, 2005). There is use of large quantity of antibiotics for human therapy, as well as for farm animals and fishes in aquaculture. The bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance plasmids. There is increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs (Hiroshi Nikaido, 2009). There are three general mechanisms studied to confer antibiotic resistance namely: prevention of interaction of the drug with target, efflux of the antibiotic from the cell, and direct destruction or modification of the compound (Wright, 2005).

To be effective, antibiotics have to accumulate in the bacterial cytoplasm. Poor permeability of outer membrane in Gram negative organisms hinders the accumulation of the drug and it pumps out the antibiotics. Gram-positive bacteria rely only to the latter mechanism of protection (Penelope and Alex, 2001).

The spread of resistant bacteria within the community increases problems for infection control (Fred, 2006).

Zoonoses are infectious diseases that can be transmitted naturally between humans and wild or domestic animals. These diseases are important in the context of emerging infectious diseases of humans. Cleaveland *et al.* (2001) identified 1,415 species of infectious organisms known to be pathogenic to humans, including 217 viruses and prions, 538 bacteria and rickettsia, 307 fungi, 66 protozoa and 287 helminths. Out of these, 868 (61%) were classified as zoonotic and 175 pathogenic species were considered to be associated with emerging diseases. Some pathogens are largely confined to animal reservoirs – human cases are infrequent or represent dead-end infections (e.g. anthrax, rabies, West Nile and Nipah/Hendra viruses), whereas others are well-established in both animals and humans (e.g. bovine tuberculosis, salmonellosis). Zoonotic diseases have relatively little impact on human health when compared to major diseases such as influenza (flu), measles, smallpox, diphtheria, or HIV/acquired immune deficiency syndrome (AIDS). It is however, increasingly clear that most of these diseases started out as zoonotic (Diamond, 2002), and the aetiology of the pandemics that have occurred during the 20th Century tend to support the notion that emerging diseases in humans originated directly from animal reservoirs rather than gradually evolving from known and existing zoonotic agents (Sllingenbergh *et al.,* 2004). Metal contamination also contributes to the maintenance and spread of antibiotic resistance factors (Craig *et al.*, 2006).

Each year several million Americans are bitten by animals, resulting in approximately 300,000 visits to emergency departments, 10,000 hospitalizations, and 20 deaths, mostly among young children (Weiss *et al.*, 1998). Ninety percent of these bites are from dogs and cats, and 3 to 18 percent of dog bites and 28 to 80 percent of cat bites become infected, with occasional consequence of meningitis, endocarditis, septic arthritis, and septic shock (Aghadabian and Conte, 1980; Callaham, 1980; Cummings, 1994; Douglas, 1975; Elenbaas *et al.*, 1984; Elliot *et al.*, 1985; Feder *et al.*, 1987; Idem, 1978; Jones and Stanbridge, 1985; Lauer *et al.*, 1982; Skurka *et al.*, 1986; Thomas and Buntine, 1987).

Bacteriologic analyses of these wound infections have focused on certain zoonotic and potentially invasive pathogens such as *Pasteurella multocida*, *Capnocytophaga canimorsus* (DF-2), and *Weeksella zoohelcum* (David *et al.*, 1999).

Dogs are human's best friend, so dogs are more closer to humans than other pet animals, that's why we choose the dog over other pet animals for this research. Dog skin and saliva contain number of micro-organisms, which may get resistant to the antibiotics, metals and other antimicrobial agents. There are chances that humans may get infected with those microorganisms, and if infection get spread into human it will be very difficult to treat these infections.

Hence, an aim of this study was to isolate and identify microflora associated with skin and saliva of dog and also to study resistance pattern of the micro-organisms isolated from dog skin and saliva.

Materials and Methods

Sample collection

Dog skin and saliva samples were collected from a private dog house, which is located at Ravet, Pune. Sample collection is done by swabbing with sterile cotton ear buds from skin and mouth of dog. Then collected samples were brought to laboratory and streaked on nutrient agar plates within 30 minutes. The dogs were of same age but of different breeds.

Isolation and characterization

Twenty five micro-organisms were isolated from dog skin and saliva sample by sub-culturing on nutrient agar plates. Morphological analysis and biochemical testing were performed such as catalase, oxidase, sugars, triple sugar iron test, oxidative fermentative test, nitrate reductase, gelatinase. The results of biochemical characterization are summarized in table 1.

Antibiotics and metals tested

Antibiotics like Amoxicillin and Ampicillin from class β-lactams antibiotics, Ofloxacin and Ciprofloxacin from class Fluroquinolones, Tetracycline and Cefafroxil from class Broad spectrum antibiotics, Metronidazole from class Nitroimidazole, Neomycin and Gentamicin from class Aminoglycosides were used.

These antibiotics were generally used to treat the dog diseases. Metals like Mercuric chloride, Nickel chloride, Magnesium chloride, Manganese chloride, Potassium chloride, Barium chloride, Ferric chloride, Copper sulphite, Cobalt sulphate and Lead acetate were tested for resistance pattern.

Antibiotic and metal resistance

Stock solution of concentration 2048µg was prepared by adding 0.01g of antibiotic and metal in 5ml distilled water separately. Antibiotic dilutions were performed by using two fold dilution scheme (Concentration ranging from 1µg to 1024µg per ml) (Raffaele *et al.*, 1997). Mueller-Hinton agar was used for checking antibiotic and metal resistance (Dhakephalkar and Chopade, 1994). The density of the inoculums was standardized so that each 1µl spot contain 10^4 CFU/ml (Andrews 2001). Mueller-Hinton plates were spot inoculated and incubated at 37^oC for 24h. Result was observed after 24h (Anand Manoharan *et al.*, 2003).

Plasmid isolation: Plasmid isolation of only Gram negative bacteria (7 isolated) from dog skin and saliva was done by Kado and Lui method (Kado and Liu, 1981).

Results and Discussion

Isolation and identification of microorganisms from dog skin and saliva:

Out of 25 isolates (Sk1-Sk13 from skin samples and Sv1-Sv12 from saliva samples) 18 isolates were Gram positive and 7 isolates (Sk11, Sv1, Sv2, Sv4, Sv5, Sv6, Sv12) were Gram negative. All isolates were capsulated. Out of 25 isolates 23 were non-motile, whereas only 2 isolates (Sk10 and Sv5) were motile. Seven isolates were identified upto Genus level namely: *Micrococcus* (Sk5) and *Planococcus* (Sk7) from skin and *Pediococcus spp.*(Sv3), *Pseudomonas spp.*(Sv5), *Alkaligens spp.* (Sv6), *Staphylococcus spp.* (Sv9) and *Deinococcus spp.* from saliva. Remaining 18 isolates were unidentified. Unidentified bacterial identification can be done in detail by 16S rRNA sequencing.

Biochemical tests for isolated microorganisms from dog skin and saliva:

All isolates showed negative result to Gelatinase. Out of 25 isolates 7(Sk6, Sk9, Sk13, Sv7-Sv9 and Sv12) isolates showed positive and 18 isolates showed negative result to nitrate reductase. All isolates were observed to be catalase positive, whereas only 20 isolates were oxidase positive and 5 isolates (Sk7, Sk11, Sk12, Sv3, and Sv8) were oxidase negative. For maltose sugar all isolates showed positive except one isolate (Sk13). Out of 25 isolates only 2 isolates (Sv1 and Sv4) do showed negative result for fructose. Other biochemical tests such as remaining sugars, TSI test and OF test are summarized in table 1.

Antibiotics and metal resistance for isolated microorganisms from dog skin and saliva:

Antibiotic resistance are summarized in table 3 and 4 for Gram-positive microorganisms and Gram-negative microorganisms respectively. For β-lactams antibiotics, seven organisms showed resistance to Amoxicillin which include Sv4, Sv5 and Sv12 Gram-negative isolates and Sk9, Sv7, Sv8 and Sv10 Gram-positive isolates. Other isolates were inhibited at different antibiotic concentration. Only 3 isolates showed resistance to Ampicillin, out of which 2 isolates Sv5 and Sv12 were Gram-negative and one isolate Sk3 was Gram-positive.

For Fluroquinolones antibiotics, two Gram-negative, Sk11 and Sv12, and ten Gram-positive showed resistance to Ofloxacin. Six Gram-positive isolates showed resistance to Ciprofloxacin antibiotic. All Gram-negative isolates inhibited at or below concentration of 512µg/ml.

For Broad spectrum antibiotics, four Gram-negative, Sv4-Sv6 and Sv12 whereas seven Gram-positive, Sk5, Sk6, Sv3, Sv7-Sv11 were resistant to Tetracycline. Other isolates inhibited at or above the concentration of 128µg/ml. Most of the Gram-positive and Gram-negative organisms are resistant to Cefadroxil antibiotic. Cefadroxil was able to inhibit four Gram-positive and three Gram-negative organisms.

In case of Nitroimidazole, Mtronidazole was able to inhibit only 2 Gram-positive organisms, all other organism showed resistance to metronidazole.

From Aminoglycosides class of antibiotics, four Gram-positive and one Gram-negative organism inhibited by Neomycin antibiotics at concentration above 128µg/ml. Other all organisms showed resistant. Three Gram-positive and four Gram-negative organisms showed resistance to Gentamicin. Other organisms inhibited at different concentration.

In case of metals, Mercuric chloride was the only metal salt which was able to inhibit 20 microorganisms out of 25. Five microorganisms (Sk6, Sv8, Sv10, Sv4 and Sv6) found to be resistance to Mercuric chloride. For other metals all microorganisms showed resistance.

Generally, organisms growing at or concentration greater than 25µg/ml were considered as resistant organisms (Dhakephalkar and Chopade, 1994).

Plasmid isolation:

Plasmid isolation was carried out only for Gram-negative bacteria, but plasmid was not isolated from any of the Gram-negative bacteria.

For this study, we have collected the samples from dog skin and saliva. From these samples 25 different microorganisms were isolated. 7 isolates were Gram-negative from genus *Agromonas spp., Alkaligens spp., Acidomonas spp., Pseudomonas spp.* 2 isolates remain unidentified. 18 isolates were Gram-positive from genus *Deinococcus spp., Micrococcus spp., Pediococcus spp., Planococcus spp. and Staphylococcus spp.* other 13 microorganisms remain unidentified. In previous study it was observed by Henning *et al.* (2001) that there are more Gram-positive bacteria on the skin as compared to the intestine where anaerobic Gram-negative bacteria are present in animals. In this study we also found more number of Gram-positive bacteria as compare to Gram-negative bacteria.

Table.1 Biochemical characterization of isolated bacteria

Strain	Catalase	Oxidase	Sugars					TSI test	OF test	Nitrate Reductase	Gelatinase
			Xylose	Rhamnose	Raffinose	Maltose	Fructose				
Sk1.	Positive	Positive	-	-	-	Acid	Acid	Yellow	Aerobic	Negative	Negative
Sk2.	Positive	Positive	Acid	-	-	Acid	Acid	Pink	Aerobic	Negative	Negative
Sk3.	Positive	Positive	-	-	Acid	Acid	Acid	Pink	Aerobic	Negative	Negative
Sk4.	Positive	Positive	-	-	Acid	Acid	Acid	-	Aerobic	Negative	Negative
Sk5.	Positive	Positive	-	-	Acid	Acid	Acid	Pink	Aerobic	Negative	Negative
Sk6.	Positive	Positive	Acid	-	-	Acid	Acid	Yellow	Aerobic	Positive	Negative
Sk7.	Positive	Negative	-	-	-	Acid	Acid	Pink	Aerobic	Negative	Negative
Sk8.	Positive	Positive	-	-	Acid	Acid	Acid	Yellow	Facultative anaerobic	Negative	Negative
Sk9.	Positive	Positive	Acid	-	-	Acid	Acid	Yellow	Aerobic	Positive	Negative
Sk10.	Positive	Positive	-	-	-	Acid	Acid	Yellow	Aerobic	Negative	Negative
Sk11.	Positive	Negative	-	-	Acid	Acid	Acid	Pink	Aerobic	Negative	Negative
Sk12.	Positive	Negative	-	-	Acid	Acid	Acid	Yellow	Aerobic	Negative	Negative
Sk13.	Positive	Positive	-	-	Acid	-	Acid	Yellow	Aerobic	Positive	Negative
Sv1.	Positive	Positive	-	-	-	Acid	-	Pink	Aerobic	Negative	Negative
Sv2.	Positive	Positive	Acid+ Gas	Acid+ Gas	Acid+ Gas	Acid+ Gas	Acid+ Gas	Yellow	Facultative anaerobic	Negative	Negative
Sv3.	Positive	Negative	Acid+ Gas	Acid	Acid+ Gas	Acid+ Gas	Acid+ Gas	Yellow	Facultative anaerobic	Negative	Negative
Sv4.	Positive	Positive	Acid	-	-	Acid	-	Pink	Facultative anaerobic	Negative	Negative
Sv5.	Positive	Positive	-	-	-	Acid	Acid	Pink	Aerobic	Negative	Negative
Sv6.	Positive	Positive	-	-	-	Acid	Acid	Yellow	Aerobic	Negative	Negative
Sv7.	Positive	Positive	-	-	-	Acid	Acid	Yellow	Facultative anaerobic	Positive	Negative
Sv8.	Positive	Negative	Acid	-	Acid	Acid	Acid	Yellow	Aerobic	Positive	Negative
Sv9.	Positive	Positive	Acid	-	Acid	Acid	Acid	Pink	Facultative anaerobic	Positive	Negative
Sv10.	Positive	Positive	-	-	Acid	Acid	Acid	-	Aerobic	Negative	Negative
Sv11.	Positive	Positive	-	-	Acid	Acid	Acid	Yellow	Aerobic	Negative	Negative
Sv12.	Positive	Positive	Acid+ Gas	Acid+ Gas	Acid+ Gas	Acid + Gas	Acid+ Gas	Yellow	Aerobic	Positive	Negative

Note: - is for Negative in this table.

Table.2 Identification of bacteria isolated from dog skin and saliva

Isolate numbers	Genus identified
Sk5.	*Micrococcus*
Sk7.	*Planococcus*
Sv3.	*Pediococcus*
Sv5.	*Pseudomonas*
Sv6.	*Alkaligens*
Sv9.	*Staphylococcus*
Sv11.	*Deinococcus*
Sk1.	Unidentified
Sk2.	Unidentified
Sk3.	Unidentified
Sk4.	Unidentified
Sk6.	Unidentified
Sk8.	Unidentified
Sk9.	Unidentified
Sk10.	Unidentified
Sk11.	Unidentified
Sk12.	Unidentified
Sk13.	Unidentified
Sv1.	Unidentified
Sv2.	Unidentified
Sv4.	Unidentified
Sv7.	Unidentified
Sv8.	Unidentified
Sv10.	Unidentified
Sv12.	Unidentified

Table.3 MIC of antibiotics resistance for Gram positive bacteria isolated from dog skin and saliva

Strain No.	Ampicillin	Amoxicillin	Ciprofloxacin	Cefadroxil	Gentamycin	Neomycin	Metranidizole	Tetracycline	Ofloxacin
Sk1.	512	32	16	R	512	R	R	64	64
Sk2.	256	32	64	R	32	R	R	128	8
Sk3.	R	1024	16	256	R	R	R	128	128
Sk4.	128	256	R	R	1024	R	R	128	R
Sk5.	64	64	32	R	R	R	R	R	1024
Sk6.	64	64	64	R	512	R	R	R	512
Sk7.	128	8	32	R	64	1024	R	512	512
Sk8.	256	16	R	R	1024	R	R	1024	R
Sk9.	256	R	16	32	1024	R	R	128	R
Sk10.	128	4	32	R	256	R	R	128	1024
Sk12.	64	128	32	64	512	R	R	128	R
Sk13.	256	256	32	R	128	R	R	256	R
Sv3.	256	128	32	R	512	R	R	R	1024
Sv7.	256	R	16	R	1024	R	R	R	R
Sv8.	512	R	R	R	R	2	32	R	R
Sv9.	512	1024	R	R	1024	R	R	R	R
Sv10.	256	R	R	128	256	8	4	R	R
Sv11.	256	1024	R	R	1024	256	R	R	R

Note: R is for Resistance and MIC units=µg/ml. More than 25µg/ml is considered as resistant (Dhakephalkar and Chopade 1994)

Table.4 MIC of antibiotics resistance for Gram negative bacteria isolated from dog skin and saliva

Strain No.	Ampicillin	Amoxicillin	Ciprofloxacin	Cefadroxil	Gentamycin	Neomycin	Metranidizole	Tetracycline	Ofloxacin
Sk11.	64	8	64	128	128	R	R	256	R
Sv1.	32	32	512	64	R	512	R	1024	1024
Sv2.	256	128	32	R	R	R	R	128	512
Sv4.	256	R	32	256	R	R	R	R	1024
Sv5.	R	R	4	R	R	R	R	R	512
Sv6.	128	R	16	R	256	R	R	R	1024
Sv12.	R	R	1024	R	256	R	R	R	R

Note: R is for Resistance and MIC units=µg/ml. More than 25µg/ml is considered as resistant (Dhakephalkar and Chopade 1994)

Table.5 MIC of metal resistance for Gram positive bacteria isolated from dog skin and saliva

Strain No.	$MgCl_2$	$MnCl_2$	KCl_2	$BaCl_2$	$FeCl_2$	$CuSo_4$	$CoSo_4$	PbCOOH	$HgCl_2$	$NiCl_2$
Sk1.	R	R	R	R	R	R	R	R	128	R
Sk2.	R	R	R	R	R	R	R	R	512	R
Sk3.	R	R	R	R	R	R	R	R	128	R
Sk4.	R	R	R	R	R	R	R	R	256	R
Sk5.	R	R	R	R	R	R	R	R	512	R
Sk6.	R	R	R	R	R	R	R	R	R	R
Sk7.	R	R	R	R	R	R	R	R	512	R
Sk8.	R	R	R	R	R	R	R	R	1024	R
Sk9.	R	R	R	R	R	R	R	R	256	R
Sk10.	R	R	R	R	R	R	R	R	256	R
Sk12.	R	R	R	R	R	R	R	R	128	R
Sk13.	R	R	R	R	R	R	R	R	1024	R
Sv3.	R	R	R	R	R	R	R	R	1024	R
Sv7.	R	R	R	R	R	R	R	R	512	R
Sv8.	R	R	R	R	R	R	R	R	R	R
Sv9.	R	R	R	R	R	R	R	R	512	R
Sv10.	R	R	R	R	R	R	R	R	R	R
Sv11.	R	R	R	R	R	R	R	R	512	R

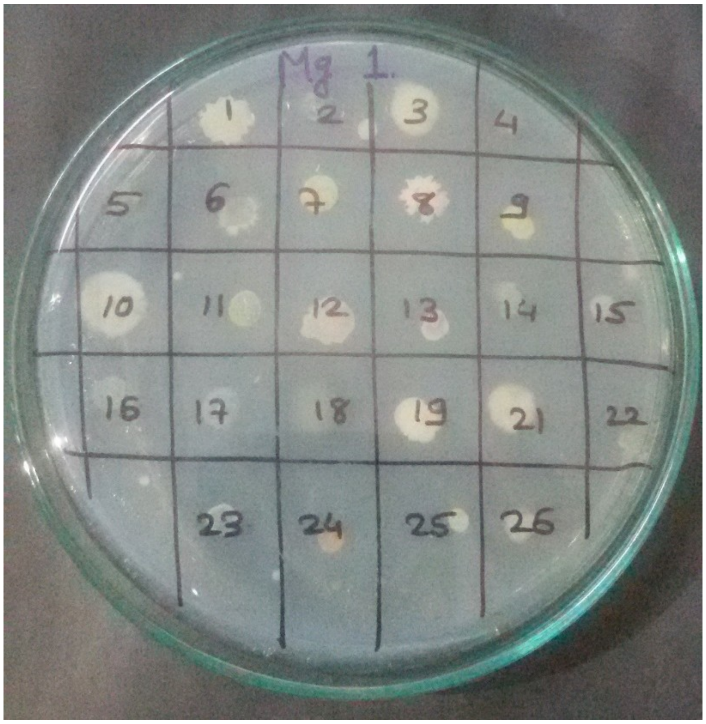
Note: R is for Resistance and MIC units=µg/ml. More than 25µg/ml is considered as resistant (Dhakephalkar and Chopade 1994).

Table.6 MIC of metal resistance for Gram negative bacteria isolated from dog skin and saliva

Stain No.	$MgCl_2$	$MnCl_2$	KCl_2	$BaCl_2$	$FeCl_2$	$CuSo_4$	$CoSo_4$	PbCOOH	$HgCl_2$	$NiCl_2$
Sk11.	R	R	R	R	R	R	R	R	256	R
Sv1.	R	R	R	R	R	R	R	R	256	R
Sv2.	R	R	R	R	R	R	R	R	128	R
Sv4.	R	R	R	R	R	R	R	R	R	R
Sv5.	R	R	R	R	R	R	R	R	128	R
Sv6.	R	R	R	R	R	R	R	R	R	R
Sv12.	R	R	R	R	R	R	R	R	1024	R

Note: R is for Resistance and MIC units=µg/ml. More than 25µg/ml is considered as resistant (Dhakephalkar and Chopade 1994)

Fig.1 Metal resistance pattern of bacteria isolated from dog skin and saliva



Detail information regarding microorganisms present on dog skin and in saliva is not available, so these results are compared with normal flora of human skin and saliva. The distribution of *Staphylococcus* and *Micrococcus* species and associated Coryneform bacteria, *Acinetobacter, Klebsiella, Enterobacter, Bacillus,* and *Streptomyces* on human skin was determined by Wesley *et al.* (1975), Patil and Chopade (2001). Our results showed similar results as we too found *Micrococcus* present on dog skin.

Froggatt *et al.* (1989) recovered *Staphylococci* from hospital environment and sources of samples were wound, urine and blood specimens. In this study *Staphylococcus* is isolated from dog saliva sample. Hence it is obvious, that dog bite may result in the Staphylococcal infections in human. There are chances of transfer on these microorganisms to humans from dog saliva.

The genus *Pediococcus* is a part of commensal intestinal flora of humans and animals and it is useful for humans (Klare *et al.,* 2007). In our study, *Pediococcus spp.* was isolated from saliva sample of dogs.

The oral cavity contains wide range of microbes which are mainly anaerobic bacteria. The predominant flora was found to be *Streptococcus viridans* (Gagliardi *et al.,* 1998). *Lactobacilli* and alphahemolytic *Streptococcus* species are frequently isolated on tonsils of healthy children (Stjernquist-Desatnik and Holst, 1999, Marushko, 1998). In present study we found that the saliva sample of dogs contains *Pediococcus spp., Pseudomonas spp., Alkaligens spp., Staphylococcus and Deinococcus spp.* As our aim was to isolate only aerobic bacteria, we have not studied anaerobic bacterial strain isolation. Hence, there is a difference between the flora isolated by Gagliardi et al.

The research papers on antimicrobial resistance in isolates from cats and dogs are very limited. Barrs *et al.* (1995) reported that there is very high number of *S. aureus* and *S. intermedius* isolated from dogs were able to produce beta-lactamase enzyme, but they were sensitive to Cloxacillin/Oxacillin. Similar results were observed in our study that *Staphylococcus* isolated from dog saliva was also resistant to β-lactams antibiotics.

Selective pressure from a metal containing environment has led to the development of resistance to all toxic metals present in soil, water and industrial waste. Both Gram-positive and Gram-negative microorganisms isolated from these sources demonstrate resistance to mercury (Bruins *et al*., 2000). But the microorganisms isolated from dog skin and saliva are in contrast are not resistant to mercury. These organisms may get transfer from soil or water source to the dog skin and saliva.

A study by Schwarz *et al.* (2001) showed that rapid spread of antimicrobial resistance between bacteria is because of the horizontal transfer of mobile genetic elements which carry one or more resistance genes. Plasmids are detected in all bacteria of medical or veterinary importance, and also in the bacteria present on skin and mucosal surfaces of human and animals. But our attempt to isolate plasmid was not successful; more elaborative investigation is needed to isolate the plasmid.

This study provides for the first time a comprehensive investigation of identification of microorganism and its resistance towards multiple drugs and metals of organisms present on dog skin and saliva. From this study it is seen that the microorganisms isolated from the dog skin and saliva are showing resistance to many antibiotics of five different classes, hence, showing multi-drug resistance. As plasmid was not isolated so may be the resistance is chromosomally coded.

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