

Isolation, Identification, Antibiotic Susceptibility and Plasmid Profiles of *Staphylococcus intermedius* Isolated from Dogs and Cats

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ملخص البحث

عند إختبار 402 عينة مسحة من الجلد والأنف والأذن لكلاب والقطط عزلت 90 معزولة (22.48%) من نوع البكتريا العنقودية المتوسطة (*Staphylococcus intermedius*) تفصيلها كما يلي: 14.83% (8/54)، 48.28% (42/87)، تم عزلها من القطط و الكلاب في العيادة الخارجية علي التوالي و 4.37% (8/183) و 41.03% (32/78) من القطط والكلاب المحجوزة بالمستشفى البيطري علي التوالي. المعزولات التسعون من العنقودية المتوسطة و التي تم عزلها من القطط والكلاب في المستشفى أو العيادة الخارجية البيطرية بواسطة اختبار حساسية الميكروبات الي 28 نمط لحساسية الميكروبات . أظهرت هذه الدراسة أن نوع البكتريا العنقودية المتوسطة أكثر تواجدا في الكلاب ، القطط و أن اختبار الحساسية للمضادات الحيوية يمكن أن يستعمل كأحد وسائل تصنيف الأنماط أما الأنماط البلازميدية (Plasmid profiles) فهي ذات قيمة تصنيفية محدودة جداً.

Summary

Out of 402 skin, nasal and ear swabs, 90 (22.48%) isolates were identified as *Staphylococcus intermedius* (*S. intermedius*). Of the 90 isolates, 14.83% (8/54), 48.28% (42/87), 4.37% (8/183) and 41.03% (32/78) were obtained from out patient cats, out patient dogs, and hospitalized cats and dogs, respectively. The 90 *S. intermedius* isolates from hospitalized and outpatient dogs and cats were typed using antibiogramer and gave 28 profiles. It was concluded that the occurrence of *S. intermedius* is more common on outpatient than hospitalised dogs and cats and the anti-biogram profiles could be useful for typing but plasmids profiles are of a very limited value.

Introduction

Staphylococci are one of the most common causes of community and hospital infections and produce a variety of disease symptoms ranging from skin pustules to bacteraemia and death. Pathogenic staphylococci are commonly found on the skin with no apparent infections,

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pneumonia and surgical-site infections in hospitalized patients (nosocomial infections). They also cause frequently blood stream infections.

Talan *et al.* (1989) have found that any coagulase positive staphylococcal isolate that give negative acetoin production test and positive β -galactosidase test was identified as *S. intermedius*. Roberson *et al.* (1992) suggested that correct identification of coagulase positive staphylococci could be obtained with growth on P agar supplemented with acriflavin and β -galactosidase tests. To facilitate the identification of *Staphylococcus* species, API system, BioMerieux SA Marcy-I'Etoile/France, developed an API STAPH strip system.

Patterns of susceptibility to antimicrobial agents are used for typing of staphylococci. However, an inherent weakness of this method is that changes in antimicrobial susceptibility are often related to environmental factors or plasmids. To enhance the value of this method as a tool for treating and typing nosocomial infections.

The importance of plasmids in antimicrobial resistance is due to their role in gene transfer among staphylococci. The transfer of genes seems more important in the spread of resistance than in the evolution of new antibiotic resistance gene. The plasmids have essential role in gene transfer, as resistance gene can migrate from a plasmid to chromosome and then back to plasmid that confers drug resistance to the other bacteria. Schwarz *et al.* (1989) found a close relationship between plasmid content and antibiotic resistance in *S. intermedius* cultures. They also found that chloramphenicol resistance in *S. intermedius* was plasmid-borne.

Plasmids were used as typing tools by many researchers. Licitra *et al.* (1989) used the plasmid analysis, susceptibility profile, bacteriophage typing and aminoglycoside modifying enzyme determinants to study dissemination of MRSA in hospitals. Greene and Schwarz (1992) evaluated antibiograms and plasmid profiles of *S. intermedius*, compared to the plasmids of other staphylococci and found marked structural homologies between them.

Here we report the occurrence rate of *S. intermedius* from hospitalised and out-patient dogs and cats and the value of antibiogram and plasmids profiles of *S. intermedius* as primary typing tools.

Materials and Methods

Samples:

402 swabs sampled from hospitalized and out patient dogs and cats, of different sex, age and breed were collected from January to September 1997. Samples were taken using sterile cotton swabs rubbed separately over the skin, ear and nose of each animal.

Isolation and biochemical tests:

The swabs were immediately streaked onto plates of Blood Agar medium, incubated aerobically at 35°C overnight and examined for colony type and morphology typical of the genus *Staphylococcus*. The colonies were subcultured onto Blood Agar and confirmed as being gram-positive and catalase producing cocci. Coagulase production was determined by tube coagulase test using rabbit plasma. Readings were made after 4 hours of incubation at 37°C and after 24 hours at room temperature. Gram positive, catalase positive, and coagulase positive cocci were identified further.

Species identification:

All coagulase positive cocci that gave a negative oxidase production and positive β -galactosidase activity tests were identified as *S. intermedius*. The API STAPH recognised the *S. intermedius* on the basis of acid formation from mannitol, acetoin production and β -galactosidase activity. The identification was facilitated by the use of a software.

Antibiograms:

These were determined by disk diffusion test using the following antimicrobial agents-containing disks: ampicillin (10 μ g), chloramphenicol (30 μ g), cephaloxin (30 μ l), cefuroxime (30 μ g), doxycycline (30 μ g), erythromycin (15 μ g), nitrofurantoin (200 μ l), gentamicin (30 μ g), kanamycin (30 μ g), methicillin (10 μ g), oxacillin (5 μ g), penicillin G (10 i.u.), streptomycin (15 μ g), tetracycline (30 μ g), and vancomycin (30 μ g), obtained from BBL Microbiology system, Cockeysville, Md, USA. Plates were inoculated and zone sizes were interpreted as described by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). The multiple antibiotic resistance index (MARI) is defined as a/b where 'a' represents the number of

antibiotics to which a particular isolate is resistant and 'b' the number of antibiotics to which the isolate was exposed (Krumperman *et al.*, 1983).

Plasmid DNA isolation:

Plasmid was extracted by the alkaline lysis method as described by Sambrook *et al.* (1989). Electrophoresis was performed in 0.8% of agarose gel. The gel was stained 0.5 µg/ml ethidium bromide, visualised under UV filter NO320 nm and photographed using Polaroid film (Type 665) and Polaroid MP-4 land camera fitted with yellow filter. Plasmids prepared by the same method for *Escherichia coli* strain V517 (Macrina *et al.*, 1978) were used as molecular size markers.

Results

Out of 402 samples examined, 90 (22%) samples yielded *S. intermedius* isolates. Of the 90 isolates, 14.83% (8/54), 48.28% (42/87), 4.37% (8/183) and 41.03% (32/78) were obtained from out patient cats, out patient dogs, hospitalized cats and hospitalised dogs, respectively (Table 1).

Table 1: Prevalence rate of *S. intermedius* in dogs and cats.

Source	No. of positive samples*				%
	Skin	Nose	Ear	Total	
Dogs in Hospital	9(26)	14(26)	9(26)	32(78)	41.03
Dogs in Clinic	17(29)	13(29)	12(29)	42(87)	48.28
Cats in Hospital	1(61)	2(61)	5(61)	8(183)	04.37
Cats in Clinic	2(18)	4(18)	2(18)	8(54)	14.83
Total	29(134)	33(134)	28(134)	90(402)	22.48

* Nos. of samples examined are in brackets.

Resistance to Antimicrobial Agents:

Out of the 90 *S. intermedius* isolates 34 (37.8%), 7 (7.8%), 4 (4.4%), 21 (23.3%), 4 (4.4%), 4 (4.4%), 12 (13.3%), 5 (5.6%), 7 (7.8%), 24 (26.7%), 14 (15.6%) and 41 (45.6%) were resistant to ampicillin, chloramphenicol, cefuroxime, erythromycin, nitrofurantoin, kanamycin, methicillin, oxacillin, penicillin G, streptomycin and tetracycline, respectively. None of the *S. intermedius* isolates were resistant to gentamicin, vancomycin and cephaloxin (Table 2). Multiple antibiotic resistances (MAR) are shown in 31 (34.4%) indexes of the strains (Table 3) that ranged from 0.24 to 0.59. The antibiotic sensitivity test gave 28 different drugs susceptibility patterns for *S. intermedius* isolates.

Plasmid Analysis:

The plasmid profiles of the ninety isolates are shown in Table 4. Forty-two (46.7%) isolates contained plasmid bands. The small plasmid bands that occurred within the strains ranged from 1.4 to 4.8 mDa.

Discussion

The data presented in the current study confirmed that the major coagulase-positive *Staphylococcus* species in dogs, in this area in Malaysia, is *S. intermedius*. Our data are similar to those of other researchers. Biberstein *et al.* (1984) found that the major coagulase positive *Staphylococcus* in dogs is *S. intermedius*. Moreover, Talan *et al.* (1989), in 135 dogs, found that *S. intermedius* was more commonly obtained as a gingival flora of the upper front teeth than *S. aureus*.

Table 2: Antibiotic susceptibility of *S. intermedius* isolates with and without DNA plasmids.

Antibiotic tested	No. and (%) of resistant isolates	No. of resistant <i>S. intermedius</i> isolates	
		P+ (42)	P- (48)
Ampicillin	34 (37.8%)	12 (28.6%)	22 (45.8%)
Chloramphenicol	7 (7.8%)	6 (14.3%)	1 (2.1%)
Cephaloxin	NR	NR	NR
Cefuroxime	5 (5.6%)	4 (9.4 %)	1 (2.1%)
Doxycycline	21 (23.3%)	10 (23.8%)	11 (22.9%)
Erythromycin	3 (3.3%)	NR	3 (6.3%)
Nitrofurantoin	4 (4.4%)	4 (9.5%)	NR
Gentamicin	NR	NR	NR
Kanamycin	12 (13.2%)	8 (19.0 %)	4 (8.3%)
Methicillin	5 (5.6%)	5 (11.9 %)	-
Oxacillin	7 (7.8%)	6 (14.3 %)	1 (2.1%)
Penicillin G	24 (26.7%)	7 (16.7 %)	17 (35.4%)
Streptomycin	16 (17.7%)	10 (23.8 %)	6 (12.6%)
Tetracycline	41 (44.4%)	19 (45.2 %)	20 (45.8%)
Vancomycin	NR	NR	NR

P+, with plasmid; P-, without plasmid; NR, no resistance

Staphylococcus intermedius was also isolated from unhealthy and healthy cats. This is in agreement with Igimi *et al.* (1994) and Hollis *et al.* (1986). The findings confirmed that the most important canine pathogenic *Staphylococcus* is *S. intermedius*. They are similar to those obtained in

pathogenicity studies in dogs and cats and in susceptibility to antimicrobials (Lavy *et al.*, 1995). our findings also show that ampicillin, streptomycin and tetracycline are not recommended for the treatment of canine skin problems caused by this *Staphylococcus*; this finding agrees with Piriz *et al.* (1996).

Table 3: Multiple antimicrobial resistance (MAR) and occurrence of plasmids in *S. intermedius* isolates, on the four groups of dogs and cats in Malaysia.

Source		MAR Indexes isolates*	Plasmids positive isolates	Plasmids negative isolates
Hospitalized (n=32)	dogs	8 (25.0%)	15 (46.9%)	17 (53.1%)
Outpatient (n=42)	dogs	20 (47.6%)	19 (45.2%)	23 (54.8%)
Hospitalized (n=8)	cats	3 (37.5%)	5 (62.5%)	3 (37.5%)
Outpatient (n=8)	cats	-	3 (37.5%)	5 (62.5%)
Overall		31 (%)	42 (46.7%)	48 (53.3%)

*isolates exhibit multiple resistance towards the antimicrobials tested.

Table 4: Plasmids DNA occurrence in *S. intermedius* isolates in different groups of dogs and cats in Malaysia.

Source	No. of plasmids / isolate					
	No. Plasmid positive isolates	1	2	3	4	5
Hospitalized dogs	15	4	6	3	1	1
Outpatient dogs	19	3	11	1	4	-
Hospitalized cats	5	2	2	-	1	-
Out-patient cats	3	-	3	-	-	-
Overall	42(46.7%)	9(21.4%)	22(2.4%)	4(.5%)	6(14.3%)	1(2.4%)

However, gentamicin and vancomycin showed excellent activity against all the isolates and could be used for the treatment of methicillin resistant *S. intermedius*. They were recommended for the treatment of otitis externa by Lopes *et al* (1990) and Kiss *et al.* (1997).

Chloramphenicol, cephaloxin, cefuroxime, methicillin and oxacillin showed a good activity; only one to three isolates were resistant. Therefore, they should be recommended for treatment of this *Staphylococcus* (Piriz *et al.*, 1995). Five methicillin-resistant *S. intermedius* isolates were recovered. Four of them from two hospitalized dogs skin and nose; they had the highest MAR index (0.59) and they harboured three to five plasmids ranging between 1.4 and 4.8 mDa. These results indicate that the methicillin-resistant *S. intermedius* had originated from high source of contamination.

All methicillin-resistant *S. intermedius* isolates carry plasmids that were similar in size to those described by Coia *et al.* (1988). There is a possible correlation between the occurrence of plasmid and the methicillin resistance. Our findings are similar to those of McDougal and Thornsberry (1986) who found that methicillin-resistant *S. aureus* strains are also resistant to the other penicillinase resistant penicillins, such as: ampicillin, penicillin, oxacillin because they produce beta-lactamase, which can hydrolyze these antimicrobial agents.

The very small plasmid bands that occurred had molecular weights ranging between 1.4 and 3.8 mDa. These types of small plasmids were previously found and evaluated by Greene and Schwarz (1992). However, no particular plasmid profile was predictive of a particular pattern of antimicrobial susceptibility. The data show that plasmid profiling is of limited epidemiological value, no correlation was found between these resistance antimicrobials and the occurrence of plasmids. However, for chloramphenicol the results of this study were similar to those of Schwarz *et al.*, (1989) who found that *S. intermedius* resistance to chloramphenicol is plasmid-borne as those of *S. aureus* and *S. epidermidis*. Similarly, Greene and Schwarz (1992) evaluated the small plasmids antibiotic resistant in *S. intermedius* and found that chloramphenicol resistance is plasmid-encoded resistance.

It is now accepted that the most important frequently occurring canine pathogenic *Staphylococcus* is *S. intermedius*. These comparative data suggest that a great proportion of bacterial isolates from hospitalised and outpatient dogs are resistant to antimicrobial agents commonly used in the control of *S. intermedius*. These data also prescribe that ampicillin, streptomycin and tetracycline cannot be recommended for the treatment of

canine skin problems caused by this *Staphylococcus*. This emphasizes the need to include laboratory tests for the determination of antibiotic sensitivity patterns of these bacterial isolates from dogs and cats.

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