

## Diagnosis of Recurrent Pyoderma in Dogs by Traditional and Molecular Based Diagnostic Assays and Its Therapeutic Approach

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**Abstract:** Canine recurrent pyoderma is a common skin problem encountered in small animal practice and also resistant staphylococci may cause hazards in contact human. The main objective of the present investigation was to study the underlying etiologies of recurrent pyoderma and antimicrobial resistance guidelines of staphylococci on traditional and molecular basis. Also, the present workup was aimed to select satisfactory antimicrobial prescriptions for cases of recurrent pyoderma on empirical and bacteriological basis. A total number of 44 dogs were thoroughly examined for dermatological lesions and classified into 32 empirically treated dogs and 12 treated dogs based on bacteriological results. Pyoderma were classified into surface (13.6%), superficial (66%) and deep pyoderma (20.5%) with main clinical signs of pruritus, skin lesions (papules and pustules), marked alopecia (specially in superficial and deep pyoderma) and epidermal collarettes. The common recurrent pyoderma was German Shepherd pyoderma (38.6%) and the common pathogen was *Staphylococcus intermedius* (100%). *S. intermedius* was isolated alone in 58.3% and 41.7% in combination with *Corynebacterium spp.* and *Staphylococcus aureus* from skin of 12 examined dogs. The present study was recorded multidrug resistance exhibited by 75% of the 12 *S. intermedius* isolates. Oxacillin MIC testing revealed 6 *S. intermedius* isolates (50%) to be resistant, which included 2 strains with the *mecA* gene. The *mec A* (Methicillin resistant *Staphylococcus intermedius*, MRSI) was detected by PCR in 5 isolates (41.7%). Amoxicillin-clavulanic acid, cephalosporines and flouroquinolones were achieved magic results on empirical and antibiogram basis in treatment of idiopathic recurrent pyoderma. It was concluded that our data provided the first Egyptian guidelines in companion animals for common bacterial pathogens with antibiogram for bacterial resistance and Antimicrobial therapy with selected antibiotics and suitable period for treatment of each type of pyoderma.

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### 1. Introduction:

Canine pyoderma is one of the most common diseases. Pyoderma literally means pus in the skin and can be caused by infectious, inflammatory and/ or neoplastic etiologies; any condition that results in the accumulation of neutrophilic exudates can be termed pyoderma. Most commonly, however, pyoderma refers to bacterial infections of the skin. Pyodermas are common in dogs and less common in cats (Leib and Monroee, 1997; Frank *et al.*, 2003; Loeffler, 2005 and Morris, 2010). Pyoderma classified according to the depth of infection into surface, superficial and deep pyodermas. Surface pyoderma are those infections that restricted to the surface of the skin and not extend into the follicle, it does not extend deeper than the stratum corneum or into hair follicle. Superficial pyodermas include infections that involve the hair follicle but do not extend into the dermis. Deep pyodermas are infections that extend into the dermis and underlying panniculitis (Ihrke, 1996; and Leib & Monroee, 1997). Pyoderma should be suspected if

the patient has a history of pruritus, especially if the pruritus has previously responded to antimicrobial therapy. The presence of papules, pustules and epidermal collarettes should create a high index of the suspicion for pyoderma. Epidermal collarettes are circular scale- crust lesions that represent the end stage of pustule (Carlotti, 1996; Leib and Monroee, 1997; and Scott *et al.*, 2001).

Pyoderma is caused most frequently by *Staphylococci*. Bacterial pyoderma is usually triggered by an overgrowth/ over colonization of normal resident or transient flora. Most canine cutaneous staphylococcal infections occur as secondary infections to predisposing factors such as atopic dermatitis (AD), flea allergy dermatitis, demodicosis or hypothyroidism (Gross *et al.*, 2005), immature immune system, such as in young animals or in those taking steroids precipitate to pyoderma. Animals with short coats, skin folds, or calloused skin were anatomically predisposed to pyoderma. Trauma from grooming, scratching, or rooting in dirt or garbage was also recorded to induce pyoderma.

The German shepherd dog has a deep pyoderma that may respond to treatment only partially and frequently recurs (Miller, 1991; Mason *et al.*, 1996; Saijonmaa-Koulumies *et al.*, 1998)

*Staphylococcus intermedius* is regarded as the primary pathogen in deep pyoderma which may develop from superficial pyoderma (Gross *et al.*, 2005). *S. intermedius* is regarded as a zoonotic pathogen. A common route of invasive infection in human is through dog bite wounds and several instances of life-threatening infections of human have been reported (Talan *et al.*, 2003 and Pottumarthy *et al.*, 2004). Thus, *S. intermedius*, especially MRSA (Methicillin resistant *Staphylococcus intermedius*), represents a potential serious public health concern. *Staphylococcus intermedius* attaches to epidermal cells of healthy dogs, but shows greater adherence to epidermal cells of atopic dogs (McEwan, 2000; Simou *et al.*, 2005 and McEwan *et al.*, 2006). It is possible that AD alters the availability of cutaneous receptors for staphylococci and facilitates bacterial adherence.

Normal resident bacteria in canine skin also include coagulase negative *Staphylococci*, *Streptococci*, *Micrococcus spp.*, and *Acinebacter spp.* Transient bacteria in canine skin include *Bacillus spp.*, *Corynebacterium spp.*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Patel, 2006). Surface pyodermas included acute moist dermatitis and intertrigo. Acute moist dermatitis (hot spot or pyotraumatic dermatitis or thick coated pyoderma) was secondary to self-induced trauma from pruritus, ectoparasitism and alterations of microenvironment of the skin (Mason & Lloyd, 1990 and Asher *et al.*, 1995). The microenvironmental change was exacerbated in long or thick coated breeds and in high relative humidity or heat and is compounded by some behaviors of animals, such as swimming. It was manifested by moist, exudative areas of erythema in addition to severe pruritus. Alopecia was the result of self inflicted trauma. Intertrigo (skin fold pyoderma or frictional dermatitis) often develop in skin folds of Brachycephalic breeds, such as English and French Bull dogs and Boston terriers (facial fold pyoderma) after Lloyd & Lampion (1999). Lip-fold pyoderma occurs in Spaniel breeds, Irish Setters. Vulvar folds or animals with corkscrew tails may develop skin fold pyoderma. The Chinese Shar-Pei can develop skin fold pyoderma in almost any area of the body. Purulent exudates and classical pyoderma lesions restricted to affected areas. It is manifested by rubbing of the face, scratching, or scooting the perineal area on a carpet, depending on the area that is involved (Allaker *et al.*, 1991 and Leib & Monroe, 1997). The treatment included surgical

correction of a conformational problem in addition to pyoderma treatment.

Superficial pyodermas are include impetigo and folliculitis. Impetigo or juvenile pyoderma characterized by development of subcorneal papules and pustules. It is caused by specific immunodeficiency syndromes, inadequate nutrition, poor husbandry (e.g. crowding, inadequate housing) and stress (Valensi, 1990 and Mason & Lloyd, 1993). Staphylococci and streptococci are most frequently isolated from the lesions. Folliculitis (short coated dog pyoderma or lumpy- bumpy disease) is defined as inflammation of the hair follicle. There are three diseases in which folliculitis are a common feature: demodicosis, dermatophytosis and bacterial infections of the skin (Mason *et al.*, 1996). It is manifested by patchy or diffuse alopecia and hairs easily epilated. The large numbers of papules has led to the term lumpy- bumpy disease (Deboer *et al.*, 1990 and Leib & Monroe, 1997). Systemic antimicrobial therapy should not less than 21-28 days (Carlotti, 1996 and De Jaham, 2003).

Deep pyodermas are include furunculosis and interdigital pyoderma. Furunculosis is defined as disruption of the hair follicle as a result of any underlying disease process. The condition may be seen in any breed predisposed to folliculitis. It is commonly occur in German Shepherd (Miller, 1991 and Chabanne *et al.*, 1995). Systemic antimicrobial therapy may take from 4 weeks to several months (Carlotti *et al.*, 2004). Furunculosis is clinically similar to folliculitis. However, as the lesions progress, draining tracts, nodules, ulcerations, and lichenification of the skin are seen. It is manifested by moderate to severe pruritus and a serosanguineous blood tinged fluid from the skin in affected areas (Wisselink, 1989; Day, 1994 and Leib & Monroe, 1997). furunculosis is often classified according to the location of lesions into canine acne (face and chin), facial pyoderma, pressure point pyoderma and interdigital pyoderma (Leib & Monroe, 1997 and Curtis *et al.*, 1999). Pododermatitis or interdigital pyoderma is a form of folliculitis and furunculosis of the interdigital areas. Erythema, papules, pustules, ulcerations and fistulous tracts are found in the interdigital spaces on the dorsum of the foot and between the pads on the ventrum. In severe cases, the feet may become severely swollen, resulting in lameness. The prescapular and popliteal lymph nodes are enlarged. Foot soaks in antiseptic solutions may provide relief to some patients but are not effective as active hydrotherapy (Wisselink, 1989 and Day, 1994).

Although data on the occurrence of antimicrobial resistance for many different animal species are reported through reporting systems in the

world as European Food Safety Authority, data on the occurrence of antimicrobial resistance in bacteria from companion animals are absent or scarce. The occurrence of antimicrobial resistance in companion animals may however be of significance to human health. Considering the shared environment of human and companion animals, transfer of resistant bacteria or mobile resistance determinants between companion animals and human is likely to occur and has been indicated in some studies (Guardabassi *et al.*, 2004 and VanDuijkeren *et al.*, 2004). However, the extent to which this exchange occurs is essentially unknown. In Denmark, few reports on the occurrence of antimicrobial resistance among bacteria from companion animals have been published and most of these investigations have focussed on *Staphylococcus intermedius* (Wegener & Pedersen, 1992 and Pedersen & Wegener, 1995).

The use of antimicrobials in companion animals has received little attention and remains unregulated, whereas antimicrobial use in farm animals is regulated in many countries by guidelines or legal restrictions (Heuer *et al.*, 2005). Some antimicrobials that are widely used in animals belong to classes of antimicrobials that are regarded as critically important for use in human (e.g. cephalosporins and fluoroquinolones) after Sternberg, 1999, and the use of these antimicrobials in farm animals is restricted or prohibited in some countries (Heiene *et al.*, 2004). Long term therapy of deep pyoderma was performed by 3 regimens. Intermittent therapy was the first regimen used in cases of recurrence, used 2- 3 times annually. Pulse therapy was the second regimen achieved by treatment 2- 7 days and stopped for 5- 32 days. Slow dose or tapering was the third regimen applied by decreasing dose gradually which suitable for cephalosporines (Negre *et al.*, 2009)

Methicillin and oxacillin are members of a class of antibacterials known as the semi-synthetic penicillinase-resistant penicillins (SSPRP). Due to its superior stability *in-vitro*, oxacillin is now used by most microbiology laboratories as the surrogate for testing the susceptibility of bacteria to this entire class of antibiotics (Chambers, 1997). Even so, the term "methicillin resistant" (MR) has persisted in the common vernacular and in most scientific publications. The SSPRP class was developed to circumvent staphylococcal resistance to the first generation penicillins, which is mediated by bacterial production of penicillinase enzymes. Although the SSPRP class is unaffected by penicillinases, it is susceptible to an acquired penicillin-binding protein (PBP), known as PBP2a. This staphylococcal PBP is encoded by the *mecA* gene, which confers an intrinsic resistance to all beta-lactam antibiotics and

their derivatives (including all classes and generations of penicillins and cephalosporins) after Berger- Bachi & Rohrer (2002). Since the early 1960's, the incidence of MR has escalated within human hospital strains of *S. aureus*, and hospital-acquired MR *S. aureus* (HA-MRSA) has now become the most prevalent pathogen causing nosocomial infections of people throughout the world (Diederer & Kluytmans, 2006). The broad antimicrobial resistance patterns inherent to HA-MRSA contribute significantly to the morbidity and mortality associated with human nosocomial MRSA infection. Since pets predominantly acquire the HA-MRSA strains, retrospective studies have attempted to test the hypothesis that outcomes for MRSA-infected pets are worse than for non-resistant *S. aureus* infections (Faires *et al.*, 2010).

Transmission of *S. intermedius* between animals and veterinarians may also be present in Egypt, and might be an emerging problem for public health (Tanner *et al.*, 2000). Presently, we sought to determine whether *S. intermedius* strains was present among various cases of canine pyoderma using samples obtained from different canine races and to determine the antibiotic susceptibility of *S. intermedius* isolates. Also, the target of present study was to detect underlying etiologies and antibiotics of choice for cases of recurrent pyoderma either empirically or based on antibiotic sensitivity testing.

## 2. Materials and Methods

A total number of 44 dogs admitted to private clinics in Giza governorate were thoroughly investigated. Age, breed and sex were recorded for each examined dog. An accurate clinical history of previous treatments, routine health care, such as internal parasite control and vaccination was collected. All investigated dogs were vaccinated and were received one tablet/ 10 Kg once of Drontal® plus (50 mg praziquantel, 150 mg Febantel, 144 mg pyrantel- Embonat, made in Germany by Bayer) as internal worm prophylaxis. Physical dermatological examinations were performed by inspection of different clinical signs. All areas of the skin should be carefully palpated and visually examined. A hand lens or other magnification was often helpful to identify lesions. The veterinarian's sense of smell may helpful to identify bad odor occurred in pyoderma. The appearance of pelage (hair coat), the ease of hair removal from follicles (epilation) and the pattern of lesions on the skin should be noted. The present study was carefully recorded findings on a special dermatologic examination form containing a silhouette of a dog for recording the location of the lesions. At each visit (every 2 weeks), They were

evaluated with lesion, pruritus and body scores (Leib & Monroee, 1997 and Mueller & Stephan, 2007).

Skin scrapings were performed for all examined dogs (Leib & Monroee, 1997). Fecal concentration floatation also was performed (Thiopont et al., 1986). Hypoallergenic (Elimination) dietary trial was performed in cases of adverse reaction to food (Leib & Monroee, 1997).

The present study was classified examined dogs into:  
Group (1): Thirty two empirically treated dogs:

The present group was performed on 9 German shepherd dogs, 9 griffon dogs, 5 American Staffordshire dogs, 4 Mastiff dogs, 3 Rottweiler dogs, one Siberian Husky dog and one Mongrel dog. Sex of these dogs was 20 male dogs and 12 female dogs. The age of dogs in the present group were ranged from 5 months into 12 years.

Firstly, affected areas were clipped and sheaved for application of topical medicaments. Topical treatments selected for all cases were Betadine® shampoo (povidone iodine 7.5%, Mundi pharmaceutical company) and Fucidin® ointment (fusidic acid 2%, Leo pharmaceutical company). Topical treatment applied every 12 hours and period of treatment differed according to the form of pyoderma. Choice of systemic antibacterial differed also according to the form of pyoderma. Four cases of surface pyoderma were treated by only topical treatment for 10 days- 2 weeks as described by Saijonmaa-Koulumies *et al.* (1998) and Loeffler *et al.* (2005). Twenty three cases of superficial pyoderma were treated by topical treatment for 2 weeks- 1 month. Systemic antimicrobial was chosen for superficial pyoderma was Augmentin® capsules (clavulanic acid potentiated amoxicillin 156, 312, 487, 625, 1g., GSK pharmaceutical company) which used/ 12 hours for 2 weeks- 1 month in a dose of 10 mg/ Kg and clavulanic acid 2.5 mg/ kg . Five cases of deep pyoderma were treated by topical treatment for 4 weeks- 3 months. Systemic treatment was selected for deep pyoderma was several antibacterial. The used antibacterial shifted every 2-3 weeks. The beginning antibacterial was Augmentin® capsules (clavulanic acid potentiated amoxicillin 156, 312, 487, 625, 1g., GSK pharmaceutical company) then shifted into Rocephin® vials every 24 hours in a dose of 15 mg/ Kg (ceftriaxone 500, 1000, Roche pharmaceutical company) and then ended by Ciprocin tablets® in a dose of 5 mg/ Kg every 12 hours (ciprofloxacin Hcl 250, 500, and 750, Eipico pharmaceutical company) as mentioned by Kruse *et al.* (1996); Holm *et al.* (2002); Negre *et al.* (2007) and Mueller & Stephan (2007).

All previous treatment was beside treatment of primary cause if detected. Cases of demodectic

mange were treated by Dectomax® injectable solution every 2 weeks (10 mg doramectin/ ml, by Pfizer Egypt veterinary pharmaceutical company) in a dose of 1 ml/ 50 kg, Amitraz® solution 0.5 ml of the solution/ L of water (emulsifiable concentrate containing 125g amitraz per liter, made in Egypt by ADWIA veterinary pharmaceutical company) and Ketrax® tablets as immunostimulant one tablet/ 5 Kg (levamisol Hcl 40 mg/ tablet, by Zenc pharmaceutical company). Cases of sarcoptic mange were treated by Dectomax injectable solution® every 2 weeks and Amitraz® solution 0.5 ml of the solution/ L of water. Flea allergic dermatitis cases were treated by Dectomax injectable solution® every 2 weeks beside treatment of allergy. Allergic skin disease cases were treated principally by avoidance of putative allergens and control of pruritis. Control of pruritis was by the use of one tablet/ 5kg prednisolone tablets® by tapering regimen every 7 days (prednisolone, 5mg/ tablet by Adco pharmaceutical company). These prescriptions were selected according to Leib & Monroe (1997).

Group (2) 12 treated dogs based on bacteriological results

The present group was performed on 8 German Shepherd dogs, 2 Rottweiler dogs and 2 Labrador dogs. Sex of these dogs was 8 male dogs and 4 female dogs. The age of dogs in the present group were ranged from 3 months into 9 years.

#### Microbial isolation

Cotton swab specimens (BD BBL Culture Swabs, Becton-Dickinson, Sparks, MD, U.S.A.) were collected from the canine skin lesions and inoculated into nutrient broth, then incubated at 37°C for 24 hours. a loopfull from each broth culture was inoculated onto 5% (v/v) sheep blood agar plate, manitol salt agar, nutrient agar and cetremid agar. The plates were then incubated at 37°C for 24 to 48 hours. Presumptive identification of different colony types was made morphologically following visual evaluation, then subsequently identified using procedures that included gram stains, and when appropriate, an evaluation for production of catalase and oxidase. Additional identification criteria for bacterial isolates were based on methods described in microbiology manuals (Isenberg, 1998). Primary identification of Staphylococci was made on the basis of colony morphology, Gram staining, and conventional catalase test. The staphylococcal isolates were further tested for coagulase synthesis, lack of colony pigmentation and acetoin production with additional confirmation being done using API 20-STAPH (bioMérieux; Marcy l'Etoile, France) and polymerase chain reaction (PCR) with previously



described *S. intermedius*-specific primers (Baron *et al.*, 2004).

#### Molecular identification of isolated *S. intermedius*

Extraction of bacterial DNA was performed by suspending 3–4 colonies of freshly subcultured strains to be investigated in 180 µl TE buffer (10 mM TrisHCl/l, 1 mM of ethylenediaminetetraacetic acid (EDTA)/l, pH 8 and 8 µl lysostaphin (1.8 U/ml; Sigma, Steinheim, Germany). After incubation for 1 hour at 37°C 20 ml proteinase K (Quiagen, Hilden, Germany) was added and the suspension was incubated for 2 hours at 56°C. The DNA was subsequently isolated with Dneasy Tissue-Kit (Qiagen) according to the manufacturer's recommendations. A PCR mediated amplification of *S. intermedius* was done according to Baron *et al.* (2004) using *S. intermedius* ATCC 29663 as reference strain.

#### Antimicrobial Susceptibility Tests

Antimicrobial susceptibilities of *S. intermedius* isolates to 16 different antimicrobials from 11 classes were tested by a disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI, M2-A9) guideline. The tested antibiotics and their concentrations were ampicillin (10 µg); amoxicillin-clavulanic acid (30 µg); amikacin (30 µg); chloramphenicol (30 µg); clindamycin (2 µg); ciprofloxacin (5 µg); cefotaxime (30 µg); erythromycin (15 µg); gentamicin (10 µg); penicillin (10 units); trimethoprim-sulfamethoxazole (23.75 µg, 1.25 µg); tetracycline (30 µg), and vancomycin (30 µg) (BD BBL). In addition, the

minimal inhibitory concentrations (MICs) to oxacillin (Sigma- Aldrich, St. Louis, MO, U.S.A.), were determined by a broth microdilution method according to the CLSI guideline (M2-A9). Isolates with oxacillin MIC $\geq$ 4mg/l were classified as being methicillin resistant, and were further confirmed by PCR using primer sets targeting the *mecA* gene as MRSI.

#### Detection of the *mecA* Gene by PCR

The presence or absence of the *mecA* gene among the *S. intermedius* isolates was determined by the PCR using the *mecA*-specific primers as previously described by Zubeir *et al.*, 2007. The oligonucleotide primer sequences used for identification of the resistance gene *mecA* were initially described by Strommenger *et al.* (2003). Details of the primer sequences and thermal cycler PCR programs are summarized in Table 1. The PCR reaction mixture (50 µl) contained 0.5 µl of each primer (50 pmol/ul), 25 µl 2X master mix (Finzyme) and 19 µl deionized H<sub>2</sub>O. Finally 5 µl DNA preparation was added to the PCR reaction mixture. The reaction mixtures were then subjected to thermal cycling (Gene Amp PCR System 2400, Perkin Elmer, Germany). The presence of PCR products was determined by electrophoresis of 10 µl of reaction product in an 1.5% agarose gel (Gibco BRL, Karlsruhe, Germany) with Tris-acetate electrophoresis buffer (TAE, 4.0 mmol/l Tris, 1 mmol/l EDTA, pH 8.0) and visualized under UV light (Image Master VDS, Pharmacia Biotech, Freiburg, Germany).

**Table (1). Oligonucleotide primer sequences and PCR conditions used in the present study**

Primer	Program <sup>a</sup>	Sequence	Suspected length	References
SInuc1 SInuc2	1	CAA TGG AGA TGG CCC TTT TA AGC GTA CAC GTT CAT CTT G	125 bp	Baron <i>et al.</i> (2004)
mecA1 mecA2	2	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	532 bp	Strommenger <i>et al.</i> (2003)

<sup>a</sup>PCR program 1: 1cycle (95 °C, 240 s), 30 cycles- (95 °C, 30 s; 55 °C, 30 s; 72 °C 30 s), and 1cycle (72 °C, 420 s). 2. 1 cycle (94 °C, 240 s), 40cycle (94 °C, 30 s; 55 °C, 30 s; 72 °C 60 s), and 1cycle (72 °C, 300 s).

### 3. Results:

The clinical presentation included panorama of clinical signs which differed according to the form of pyoderma. Six cases of surface pyoderma (13.6%) manifested by erythema, scales, crusts, pruritis, weepy skin (purulent exudates) and salivary staining on rump when biting it (Fig. (I), A and B). Twenty nine cases of superficial pyoderma suffered from massive erythema, pustules, erythema, cracks, excoriations, epidermal collarettes, pruritis, alopecia, bad odour of skin and easily epilated hairs (Fig. (I), C, D, E and F). Nine cases of deep pyoderma (20.4%) showed massive alopecia all over the body, erythema, very offensive odour of skin, small abscesses and pododermatitis (Fig. (I), G and H).

The initiating etiology of empirically treated dog was illustrated in table (2). Flea allergic

dermatitis (8 cases out of 44) and Demodectic mange (8 cases out of 44), each one represented by 18.2 % as primary cause of pyoderma. Cases of flea allergic dermatitis were manifested by presence of fleas or flea dirt, intense pruritus, signs of secondary pyoderma. In demodectic mange, mostly animal kept in captivity). Cases of sarcoptic mange (3 cases out of 44) represented by 6.8% and showed signs of secondary pyoderma, crusty ears and massive itching. Allergic conditions included contact allergic dermatitis (4 cases out of 44- 9%), atopy (2 cases out of 44- 4.5%), food allergy (2 cases out of 44- 4.5%) and flea allergic dermatitis. Cases of allergic conditions were manifested by signs of secondary pyoderma and intense pruritus.

#### Figures (I): Clinical presentation of different forms of pyoderma



A-6.3 Ys old German Shepherd dog suffered from surface idiopathic pyoderma showing crusty lesion with purulent exudates (Hot spot or pyotraumatic pyoderma by self-inflicted trauma) in the skin of wither.; B-4.7 Ys old German Shepherd dog suffered from surface pyoderma displaying salivary staining as self induced trauma, moist exudative areas of erythema and detructed hairs in the skin of back (flea allergic dermatitis).; C-5 months old Bull Mastiff female puppy suffered from superficial pyoderma manifested by papules, pustules and crusts (Juvenile pyoderma or impetigo) in the skin of ventral abdomen.; D-2.9 Ys old Rottweiler bitch suffered from superficial pyoderma showing patchy alopecia, ulceration and purulent exudate (idiopathic short coated dog pyoderma) in the skin of hunch.



E-9.4 Ys German Shepherd dog suffered from superficial pyoderma displaying massive ulceration, papules, pustules, crusts and patchy alopecia (folliculitis after contact allergic dermatitis) in the skin of back.; F-6.5 Ys German Shepherd dog suffered from superficial pyoderma manifested by diffuse alopecia, large numbers of papules and pustules (Lumpy- bumpy disease).; G-3.7 Ys Neapolitan Mastiff dog suffered from deep pyoderma showing lichenification of skin and patchy alopecia in different body regions (furunculosis after demodicosis detected microscopically).; H- 2.4 Ys old Labrador dog suffered from deep pyoderma showing erythema and ulcerations (pododermatitis or interdigital pyoderma).

The form of idiopathic pyoderma in 12 dogs treated on the basis of bacteriological investigations was 2 cases of surface pyodermas, 6 cases of superficial pyodermas and 4 cases of deep pyodermas.

Types of pyoderma in both groups (1) and (2) was arranged in order where the highest

percentage was superficial pyoderma (29 out of 44 dogs- 66%), followed by deep pyoderma (8 out of 44 dogs- 20.5%) and surface pyoderma (6 out of 44 dogs- 13.6%). Idiopathic cases without underlying etiologies (17 cases) represented 38.6% of all pyoderma cases in both groups (1) and (2).



**\*Table (2): classification of cases according to depth and initiating cause of Group 1 (32 empirically treated dogs)**

Initiating cause	Surface P.	Superficial P.	Deep P.	total
FAD	one dog	7 dogs	0 dog	8
Atopy	0 dog	One dog	One dog	2
Food allergy	0 dog	2 dogs	0 dog	2
Contact allergic dermatitis	One dog	3 dogs	0 dog	4
Demodectic mange	0 dog	5 dogs	3 dogs	8
Sarcoptic mange	0 dog	2 dogs	One dog	3
idiopathic	2 dogs	3 dogs	0 dog	5
total	4	23	5	32

\*P. = pyoderma \*FAD = flea allergic dermatitis

Skin scraping revealed 8 cases of *Demodex canis* and 3 cases of *Sarcoptes* spp. While fecal examination demonstrated 8 cases of *Dipylidium caninum* egg nests.

Response to treatment in group (1) differed according to the form of pyoderma. All cases of surface pyoderma responded efficiently to topical treatment. The treatment period lasted 7-14 days. Cases of superficial pyoderma cured by topical treatment and Augmentin® within 13 days to 4 weeks without recurrence except 4 cases. The four cases suffered from demodectic mange (2 cases) and food allergy (2 cases). The used antibacterials in both conditions shifted every 2 weeks till complete cure into Rocephin® vials then Ciprocil® tablet. The response delayed in demodectic mange upto 6-7 weeks. While in food allergy was responded in 6 weeks by the elimination trial. Cases of deep pyoderma responded to topical treatment and systemic antimicrobials within 3 weeks- 13 weeks (except 4 cases). One case of generalized

demodectic mange was euthanized as it was not responded to treatment. There were 3 cases of recurrence, 2 cases of demodectic mange and one case of atopic dermatitis which delayed the treatment of deep pyoderma.

#### Results of microbial isolation

*S. intermedius*, which preliminary identified based upon their properties in culture, beta-hemolysis on sheep blood agar, a positive coagulase, a negative Voges Proskauer and the results of API 20-STAPH, was isolated from 12 samples (100%) taken from the skin during the time period under investigation and was isolated alone from the skin of 7 dogs (58.3%). It was isolated in combination with *Corynebacterium* spp. and *S. aureus* from the other samples (41.7%) as shown in the table (3); the species identity confirmed by PCR yielding positive reactions with the *S. intermedius* nuc gene specific oligonucleotide primer.

**Table (3) results of microbial isolation**

Animal species	No. of samples	<i>S. intermedius</i>	<i>S. aureus</i>	<i>Corynebacterium</i> spp.
German Shepherd	8	8 (100%)	1 (12.5%)	4 (50%)
Rottweiler	2	2 (100%)	0 (0%)	1 (50%)
Labrador	2	2 (100%)	0 (0%)	2 (100%)

#### Antibiotic resistance of *S. intermedius* isolated strains

The result of disk diffusion test is shown in Fig. 1. Resistance to more than three antimicrobial classes represented multidrug resistance (MDR); MDR was exhibited by 9 (75%) of the 12 *S. intermedius* isolates. Oxacillin MIC testing revealed 6 *S. intermedius* isolates (50%) to be resistant, which included 2 strain with the *mecA* gene that did not display oxacillin resistance in the MIC test.

#### Identification of MRSI Using *mecA* Gene PCR

PCR was performed to detect the *mecA* gene in all 12 *S. intermedius* isolates. The *mecA* gene was detected in 5 (41.7%) isolates; of these, 3 exhibited MIC-determined oxacillin resistance.



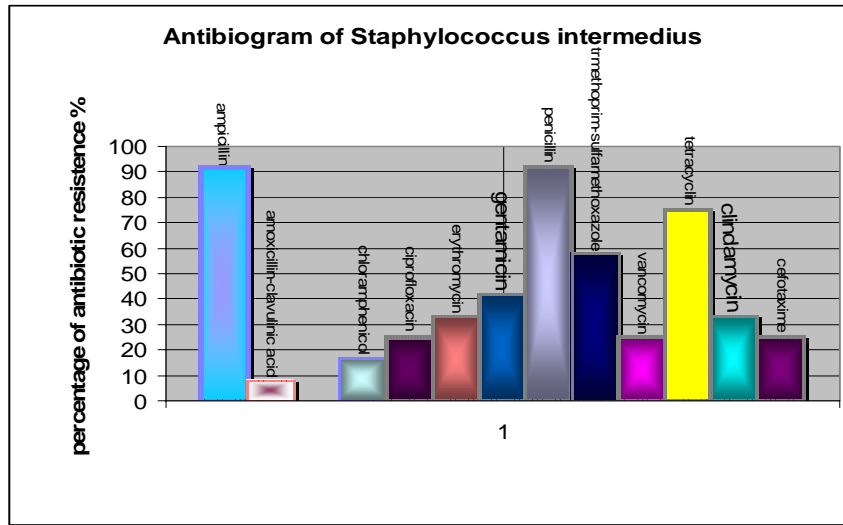


Fig. 1. Antibiogram of *Staphylococcus intermedius* isolates

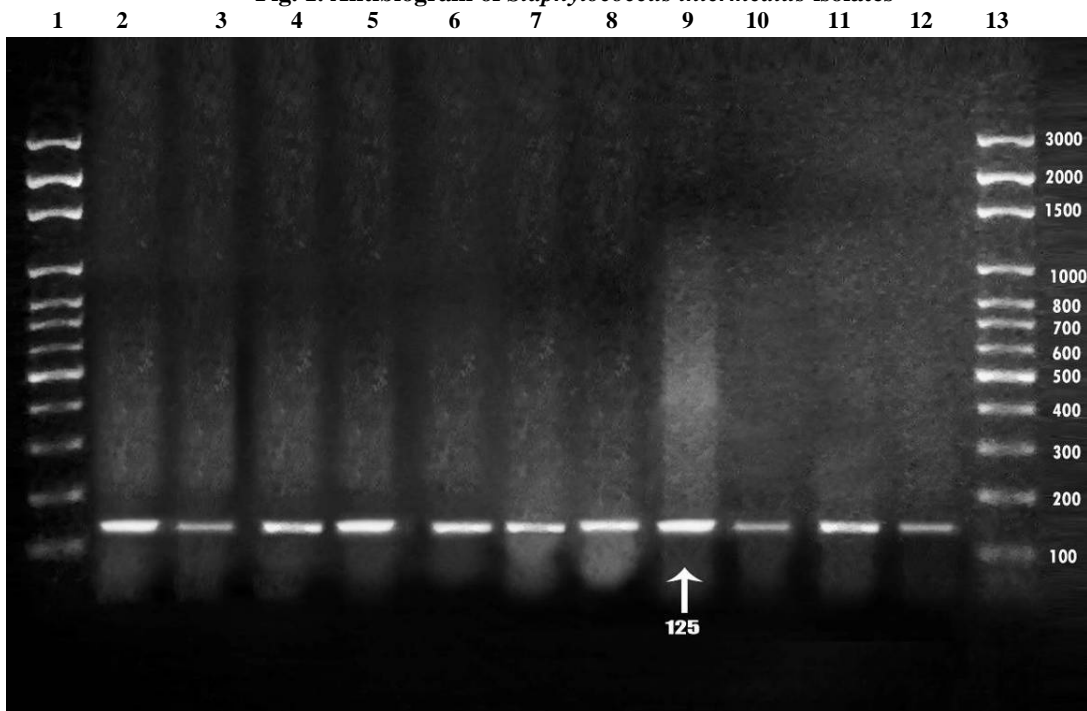
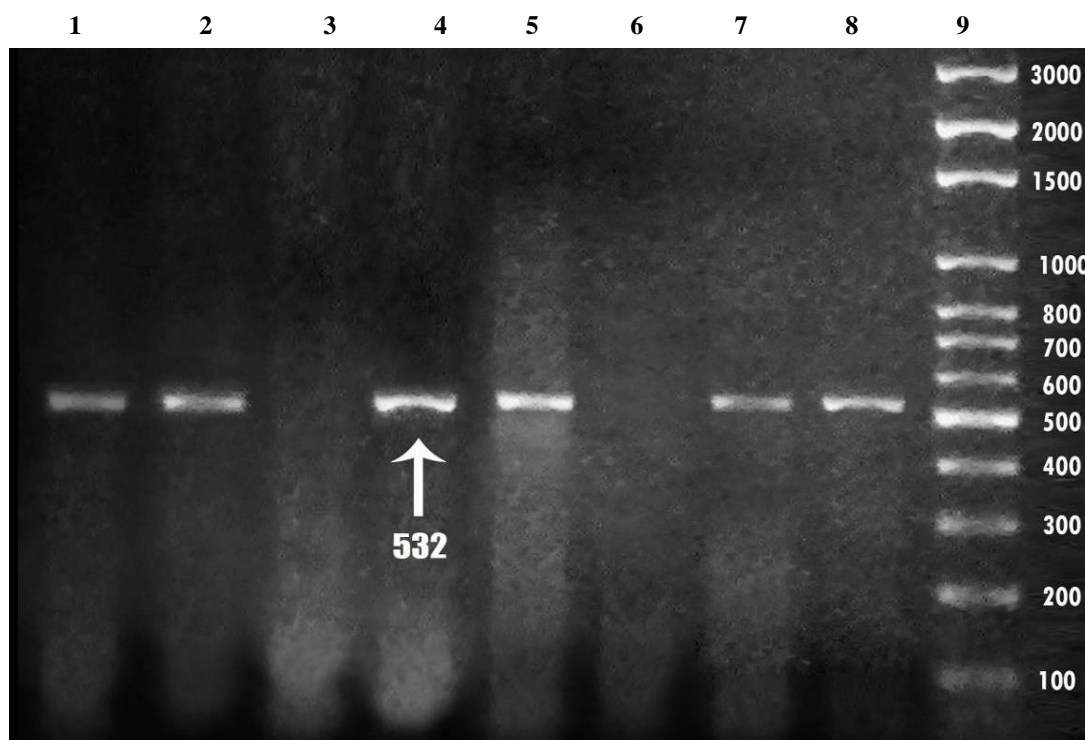


Fig. 2. PCR assay for identification of *S. intermedius*. Lanes 1&13 DNA ladder; Lanes 3-12 *S. intermedius* isolates from pyoderma cases; Lane 2 reference *S. intermedius* strain.



**Fig. 3. PCR assay for identification of methicillin-resistant *S. intermedius*. Lanes 1, 2, 4, 5 & 7 MRSA isolates; lanes 3 & 6 non MRSA isolates.**

Response to treatment in group (2):

Six cases were responded to amoxicillin-clavulanic acid within 2- 3 weeks. Four cases were treated effectively by ciprofloxacin within 3- 6 weeks. While 2 cases were treated to cefotaxione, where one case was responded within 3 weeks and the other case was responded within 5 weeks.

#### 4. Discussion:

Canine pyoderma is a complex of diseases involving bacterial infection at different levels of the skin (Table 2) and requiring different approaches to therapy. These diseases are nearly always secondary and so it is important to identify underlying factors. Commonly these are allergies but endocrinopathy, immunodeficiency, ectoparasitic infestation, follicular dysplasia and breed predisposition may be involved. Diagnosis of underlying conditions may not be easy. Treatment during the diagnostic phase was designed to advance diagnosis and to avoid camouflaging diagnostic clinical signs. Antibiotic therapy is a good diagnostic strategy as it eliminates pyoderma and helps expose underlying conditions (Scott *et al.*, 2001 and Patel, 2006).

For many reasons, clients may not permit or be able to afford all diagnostic procedures deemed appropriate by the clinician. Therefore, empiric

treatment is often prescribed by the veterinarian. Whenever treatment is administered or prescribed without a clear diagnosis, the treatment should be considered diagnostic procedure. Antimicrobials used to rule out or reduce bacterial dermatitis. The choice of antimicrobials was differed according to form of pyoderma (Kruse *et al.*, 1996; Holm *et al.*, 2002 and Loeffler *et al.*, 2005).

Pyoderma was long-standing problems that may predispose for development of resistance due to repeated or prolonged antimicrobial treatment (Guardabassi *et al.*, 2004 and Morris *et al.*, 2010). It is noteworthy that 81% of the total amount of antimicrobials prescribed for companion animals was the broad-spectrum compounds, cephalosporins, extended-spectrum penicillins (69% with clavulanic acid) and trimethoprim potentiated sulphonamides, which is much in contrast to human medical practice (Heuer *et al.*, 2005).

Primary disease processes should be considered whenever a case has been recurring pyoderma or pyoderma that does not respond to the appropriate therapy. A number of factors predisposed dogs to a bacterial infection of the skin. Underlying diseases such as ectoparasitism or flea allergic dermatitis (18.2%), atopy (4.5%), adverse reactions to food (4.5%), contact allergic dermatitis (4.5%),

sarcoptic mange (6.8%), resulted in secondary pyoderma caused by self inflicted trauma. Non pruritic conditions associated with bacterial infections include specific immunodeficiency syndromes as immunoglobulin A deficiency in German shepherd (38.6% in our study) as reported by Miller (1991) and demodicosis (18.2% in our study) as recorded by Leib & Monoroe (1997).

Dogs get pyoderma more readily than people due to the unique characteristics of dog skin. Dog skin has a thin stratum corneum with less lipid material and unprotected hair follicles that are increased risk for bacterial invasion and subsequent colonization and overgrowth. This may lead to superficial bacterial folliculitis. There is the potential for the transfer of bacterial resistance genes from drug resistant staphylococci of dogs to human pathogenic staphylococci (Divriese *et al.*, 2005 and Takashi *et al.*, 2007).

German Shepherd dog pyoderma is a deep pyoderma that has a familial tendency in the breed. The mode of inheritance is hypothesized to be autosomal recessive. The etiology is unknown, but an immunologic defect is suggested (Divriese *et al.*, 2005; Stegemann *et al.*, 2006 and Takashi *et al.*, 2007)

*Staphylococcus intermedius* is the primary pathogen of canine skin. It is a coagulase-positive, B-lactamase-producing staphylococcus (Bannoehr, 2007). The cell wall of this bacterium contains the substance protein A, which has many biological effects, including activation of complement, induction of immediate and delayed hypersensitivity reactions, lymphocyte stimulation and inhibition of phagocytosis (Cox *et al.*, 1986; Miller, 1991 and Day, 1994). Staphylococci also produce a variety of enzymes that may participate in the pathological processes (Leib & Monoroe, 1997 and Scott, 2001). Although coagulase-positive Staphylococci are not considered part of the resident microflora of canine skin, they are consistently present on normal canine hair (Allaker, 1991 and McEwan *et al.*, 2006). Adherence of bacteria to the epithelial cells is required for bacterial colonization, environmental (temperature and humidity), host (underlying diseases) and organisms characteristics affect adherence.

Six cases of surface pyoderma was represented by 13.6%. Acute moist dermatitis was attributed to bacterial proliferation on the skin and subsequent release of bacterial toxins and enzymes resulted in inflammation and pruritus. As the animal traumatizes the skin in response to the pruritus, The infection became more severe and a vicious circle of pyoderma and pruritus was established (Leib & Monoroe, 1997 and Loeffler *et al.*, 2008).

Twenty nine cases of superficial pyoderma represented by 66%. Folliculitis was occurred as follicle growth cycle shifted to the telogen phase after inflammation occurs in and around the follicle. Hairs were the easily epilated, resulting in diffuse or patchy alopecia (Leib & Monoroe, 1997 and King *et al.*, 2006).

Seven cases of deep pyoderma (Furunculosis- 20.5%) was the end stage of untreated cases of folliculitis. When furunculosis occurred, the infectious agent responsible for folliculitis and the keratinized structures of the follicle (e.g., hair) were released into the surrounding dermis. The result was a deep infection of the skin and secondary foreign body reaction in the dermis. Draining tracts developed if the follicle was destroyed (Leib & Monoroe, 1997). High percentage of superficial and deep pyoderma in our work-up was attributed to carelessness of companion animal owners about the dose and duration of antimicrobials. When the pets achieved clinical remission, the owners stopped the treatment. So, the bacterial resistance and multi-drug resistance were the end result. Response to treatment in our study by tapering method using amoxicillin-clavulanic acid, cefotaxione and ciprofloxacin was within 3-13 weeks except 3 cases of demodicosis and one case of atopy. Also results of Mueller & Stephan (2007) indicated that pradofloxacin as a member of fluoroquinolones was an efficacious therapy comparable to amoxicillin-clavulanic acid for deep bacterial pyoderma in dogs. While Negre *et al.* (2009) recorded that deep folliculitis or furunculosis was treated within 4-6 weeks and in some cases of scarred granulomatous lesions was cured within 3-6 months.

Pododermatitis was associated with foreign material (e.g. plant awns), bacterial folliculitis, trauma, atopy, adverse reaction to food, demodicosis and contact allergic dermatitis (Leib & Monoroe, 1997). The bacterial component of the pododermatitis may be a primary factor or an opportunistic infection (King *et al.*, 2006 and Bannoehr *et al.*, 2007).

With discharging lesions, antimicrobial washes and soaks were useful to remove pus and debris, and were accelerated the recovery. Clipping was helpful, enables the extent of lesions to be demonstrated and can be useful in persuading clients to comply with treatment. Prolonged systemic antibiotic treatment with bactericidal antibiotic was necessary and was continued for at least two weeks beyond clinical cure. Where lesions were in areas with poor blood supply or large granulomatous lesions, fluoroquinolones, which penetrate well, are particularly useful. Plant *et al.* (1992); Rosenkrantz (2006) and Loeffler *et al.* (2008) also reported that cleaning of the skin and topical therapy in some cases

2-3 times a week was very effective in preventing recurrent pyoderma and bacterial overgrowth and yeast as well as irritants and allergens. In addition to moisturizing the skin resulted in decreasing of pruritus. For MRS, daily therapy was needed and generally was best with shampoos but sprays and rinses may also be effective.

Despite the reported escalation of methicillin resistance (MR) in staphylococci of veterinary origin, the majority of staphylococcal strains residing on dogs and cats continued to be susceptible to most classes of antibiotics, including the beta-lactams. Therefore, empirical therapy of first-time skin and soft tissue infections with “pet friendly” drugs such as amoxicillin-clavulanic acid, cephalosporins, and clindamycin continued to constitute acceptable practice (Abraham *et al.*, 2007; Griffeth *et al.*, 2008 and Morris, 2010). The absence of resistance to amoxicillin with clavulanic acid in this study is encouraging, albeit surprising, since this drug combination for many years has been one of the most often prescribed antimicrobials for dogs, in particular for skin infections, due to the very frequent resistance to penicillin (Heiene *et al.*, 2004).

To our knowledge, this study was the first published analysis of the antibiograms of *S. intermedius* isolated from dogs in Egypt. 12 *S. intermedius* isolates (8 German Shepherd, 2 Rottweiler, and 2 Labrador) were isolated in a percentage of 100%, which was a higher isolation rate than that reported in a previous study (Morris *et al.*, 2006 and Youn *et al.*, 2010). The reasons for the high rate of *S. intermedius* isolation from the skin may be due to the samples were collected from wound area and *S. intermedius* is a temporary bacterium on the skin and hair coat of dogs (Hartmann *et al.*, 2005). The high antibiotic resistance of *S. intermedius* isolates against penicillin and ampicillin (Fig. 1) might be due to the frequent use of penicillin G and ampicillin in small animal hospitals in the past few years in Egypt. The results of the disk diffusion test of *S. intermedius* isolates from the present study were different from previous 2 studies (Boerlin *et al.*, 2001 and Loeffler *et al.*, 2007) while they were agree with that obtained by another previous study (Youn *et al.*, 2010). All four studies showed high resistant rates against penicillin, whereas the *S. intermedius* isolates for example displayed 100% resistance against amoxicillin-clavulanic acid (Loeffler *et al.*, 2007) which is markedly higher compared with resistance rates of 8.3% determined presently and the rate of 2.74% and 1.37% determined in the other studies (Boerlin *et al.*, 2001 and Youn *et al.*, 2010, respectively). These differences might be due to the variation in the sampling source (wound area versus wound-free

area), diverse selective pressure exhibited by the use of different antibiotics in different regions (Germany, Switzerland, Korea and Egypt), and sampling sources. Presently, oxacillin MIC results indicated a resistance rate of 50%, which is markedly different from the rate of 0% reported by five other studies. The occurrence of *S. intermedius* resistant to all antimicrobials commonly used for systemic therapy in small animal medicine is alarming. This is of special concern, since *S. intermedius* has so far not presented as a therapeutic problem in pets in Egypt and resistance to several antimicrobial classes appears rare (Ganiere *et al.*, 2005; Lloyd *et al.*, 1996 and Manian, 2003). In addition, a total of 5 *S. intermedius* isolates (41.7%) possessed the *mecA* gene and thus the abbreviation MRSI is appropriate in analogy to MRSA.

In conclusion, the emergence of MRSI in Egypt as a cause of canine skin infection is alarming and this highlights the importance for vigilance by hospital staff, who may serve as carriers for the pathogen. To prevent transmission and avoid the outbreak of disease caused by *S. intermedius* and MRSI, prudent use of antibiotics and strict infection control practices in animal hospitals should be enforced. In addition, continuous monitoring and molecular epidemiological studies should be followed. As the animals spend the main time with their owner and in their homes, continuous sampling of these two groups will give us more information about the spread and antibiograms of *S. intermedius*. The prudent strategic use of antimicrobials, reserving systemic application for deeper and more complicated infections, may limit the spread of multiresistant staphylococci in patients with skin disease. With antimicrobial resistance as one of the most pressing public health problems for the future, the veterinary profession needs to be proactive in monitoring and controlling antimicrobial resistance and use in small animals at the local, national and international level. Advice on the zoonotic potential of staphylococci is important when dealing with multidrug resistant isolates and for immunocompromised owners or those undergoing surgery and this should only be assessed by medical doctors together with their full medical history.

## 5. Conclusion:

Canine recurrent pyoderma is a group of various skin diseases and an accurate diagnosis is mandatory. In the cases of broadly drug-resistant pyoderma, intensive topical therapy is often beneficial as either the sole treatment for localized pyoderma, or as an adjunct to systemic treatment of more generalized disease. Antibiotics must be prescribed carefully at an adequate dose for an



appropriate duration. This investigation provided data on occurrence of antimicrobial resistance in important pathogenic bacteria from dogs as the first record in companion animals in Egypt, which may be useful for the small animal practitioner. Resistance was low to the compounds that were most often used, but unfortunately, these compounds were broad-spectrum. Data on resistance and usage may form a background for the establishment of a set of recommendations for prudent use of antimicrobials for companion animals.

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