Conference paper
Izlaganje sa znanstvenog skupa

STAPHYLOCOCCAL INFECTIONS IN POULTRY

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Abstract

The genus *Staphylococcus* includes important commensals and (opportunistic) pathogens in humans and animals. Most commonly, they can be found on the skin and mucosae of birds and mammals. Staphylococcosis is either systemic infection characterised by gangrenous dermatitis and septicaemia or localized infection (pododermatitis, arthritis, tenosynovitis, osteomyelitis, omphalitis). Poor immune status of the host, as in other opportunistic infections, facilitates development of the disease. In poultry production, staphylococcosis may cause significant losses. Currently, prevention is the best cure since there is no effective vaccine against this pathogen. In addition, antimicrobial resistance continues to be the problem in veterinary medicine and includes treatment of staphylococcosis. Development of new technologies has led to new findings on its pathogenicity and resistance, although lack of data is still hindering the development of new effective treatments. Therefore, maintenance of healthy poultry population will assure not only safe food supply but would also lower the risk of unwanted infections in humans, as well as transmission of this pathogen in the environment. Further research is necessary to define the zoonotic potential of staphylococcal species and provide an alternative way for treatment of resistant strains.

Key words: *Staphylococcus*, infection, poultry, resistance, staphylococcosis

Introduction

Staphylococcus bacteria are gram-positive aerobic, facultative anaerobic organisms that have coccoid shape. First description of staphylococcal species dates back in the late nineteenth century, in the time of Louis Pasteur, as one of the first described pathogens. They were classified according to Gram-staining, coagulase, catalase (slide agglutination) and serological testing. For a long time, all gram-positive catalase producing cocci that grow and produce acid from glucose were classified as *Staphylococcus* species (Baird-Parker, 1965). Additional schemes were introduced by several other researchers and bacteria were classified in the genus *Staphylococcus* if they were gram-positive cocci visible as grape-like clusters (under microscope) (Figure 1), produce catalase and have G-C content of 30-40 mL%. Further classification into species depended upon growth requirements, oxidative and fermentative activity on carbohydrates, haemolytic activity (on sheep or bovine blood agar), susceptibility to novobiocin and enzymatic reactions (i.e., biochemical profiling) (Figure 2). According to the capsular polysaccharide antigen, they can be classified in several types of which types 5 and 8 are most common

Rad je prezentiran na XV. simpoziju Peradarski dani 2024., Pula, Hrvatska, 22. – 25. svibnja 2024. te objavljen u Zborniku radova.

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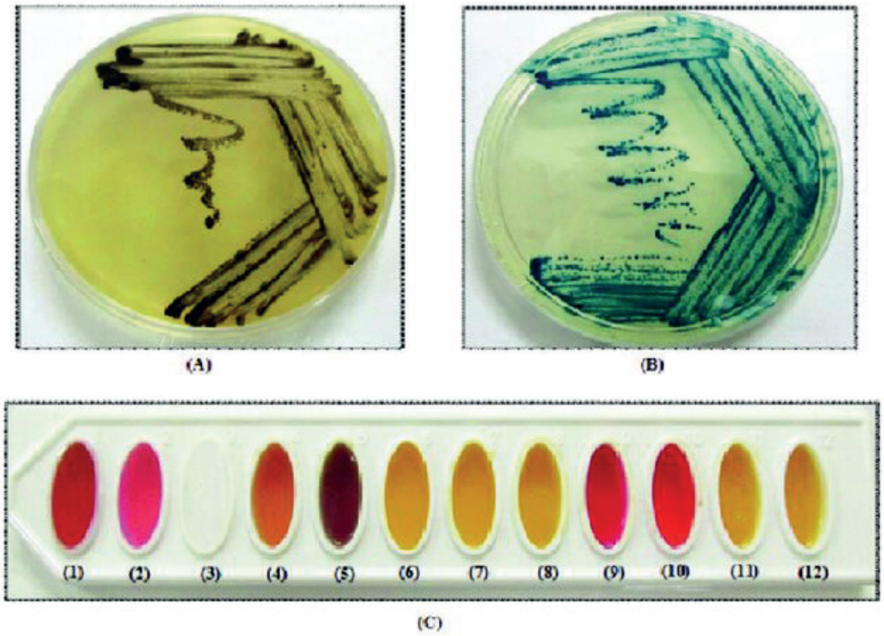
in isolates from poultry (Kloos and Schleifer, 1975; Daum *et al.*, 1994; Freney, 1999; O'Riordan and Lee, 2004). Among all the species, *S. aureus* attracted greatest attention and the main task was to differentiate it from other species (thermonuclease and coagulase tests, latex agglutination, lysostaphin susceptibility, *etc*.). Development of molecular methods enabled more detailed and precise identification of this pathogen. Currently, the genus *Staphylococcus* includes 88 species and 30 subspecies (Parte *et al*., 2020) and the genome sequence of an *S. aureus* strain is known since 1996 (Proctor, 2016). Although pathogenicity can be evaluated with molecular methods capable of detecting multiple factors necessary for the phenotypic expression of traits (e.g., methicillin resistance), phenotypic tests are still used in diagnostics of this species and especially for identification of the life-threatening methicillin resistant *Staphylococcus* aureus (MRSA) strains (Sato *et al*., 2009; McCallum *et al*., 2010; Proctor, 2016; Gaillot, 2000). The aim of this paper is to provide an overview of staphylococcosis and problems that these bacteria can cause in poultry.

Figure 1. Microscopic and phenotypic representations of *Staphylococcus aureus*: (A) grape-like (circular) clusters; (B) gold colonies displayed on enriched culture media (FAQ, 2015).
(A) Gram positive, cocci, *Staphylococcus* sp. Visual Resources. American Society for Microbiology, (B) Large, creamy white, beta hemolytic colonies typical of *Staphylococcus aureus*.



(A) Growth of coagulase positive *S. aureus* in Baird Parker media with egg yolk and sodium terrulite supplementation; (B) growth of MRSA in Hichrome MeReSa Agar Base using MeReSa Selective Supplement; (C) biochemical tests as performed by HiStaph TM Identification. (Kit-Well 1: Voges Proskaeuer's test-(+), Well 2: Alkaline Phosphate-(+), Well 3: ONPG-(-), Well 4: Urease-(+), Well 5: Arginine utilization-(+), Well 6: Mannitol-(+), Well 7: Sucrose-(+), Well 8: Lactose-(+), Well 9: Arabinose-(-), Well 10: Raffinose-(-), Well 11: Trehalose-(+), Well 12: Maltose(+).

Figure 2. Identification of *S. aureus* isolate (from: Biswas *et al.*, 2018).

Sources

Bacteria from the genus *Staphylococcus* are ubiquitous organisms that can be both commensals or pathogens. As commensals, they can be found on the skin and mucosae of humans and animals, but can also be found in the soil, water, dust and air. It is estimated that in dry environment, some strains can survive up to several months and show resistance to heat and certain disinfectants (Mead and Adams, 1986; Andreasen, 2020). In respect to epidemiological and public health significance, high numbers of these pathogens have been found in hospitals, private and public places, wastewater treatment plants, and livestock husbandry. For example, in poultry farms, it was demonstrated that *S. aureus* was present in the air (23-51 CFU/m^3) and that the potential origin of these strains was chicken faeces (Zhong *et al.*, 2009). In a similar research, Liu *et al*. (2012) also confirmed the potential transmission routes of *S. aureus* strains between poultry farms and the environment. In addition, they isolated 149 strains of which 15 types exhibited antibiotic resistance and eight of them were methicillin resistant. Similar results were found at other animal farms (Kozajda *et al*., 2019). These results indicate the potential dissemination route of pathogenic bacteria between faeces and bioaerosol within poultry houses as the possible sources of infection in humans (farm workers), as well their spread in the environment.

Staphylococcal species in animals

All staphylococcal species can be commensals in animals, including poultry, and all members of this genus are opportunistic pathogens. However, pathogenic strains mostly belong to the coagulase-positive staphylococci (CoPS) group, and the prominent pathogenic member *S. aureus* is the most often reported pathogen in poultry. Recently, molecular approach in the identification of staphylococci has enabled detection of a great number of other pathogenic species in animals, including pathogenic strains that belong to coagulase-negative staphylococci (CoNS), methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. pseudointermedius* (MRSP) (Devriese *et al.,* 2005; Weese and van Duijkeren, 2010). As opportunistic pathogens in poultry, often in combination with other bacteria isolated were *S. lentus, S. simulans, S. cohnii, S. gallinarum* and *S. capitis* (Andreasen, 2020). *S. hyicus* was isolated as the cause of fibrinoheterophilic blepharitis in chickens and turkeys and chronic folliculitis and epidermitis with acantholysis in laying hens (Chénier and Lallier, 2012). Scabby hip lesions in broiler chickens were caused by several staphylococcal species, i.e., *S. sciuri, S. simulans, S. epidermidis, S. lentus, S. warner, S. cohnii* and *S. intermedius. S, agnetis* was correlated with femoral head necrosis in chickens and *S. simulans* with endocarditis (Awan and Matsumoto, 1998; Stepien-Pysniak *et al*., 2017; Andreasen, 2020; Szafraniec *et al*., 2020).

Virulence factors and pathogenesis

Staphylococci have numerous virulence factors that enable their colonization and persistence in the host organism, development of infections, invasive diseases and septicaemia. The most important virulence factors that contribute to the pathogenesis include coagulase (clot formation in blood plasma that prevents host immune response), adhesins (facilitate adhesion and formation of biofilms), capsular polysaccharide (interferes with immune recognition and phagocytic killing), peptidoglycan and other cell wall components (enable long-term colonization and reduction of immune response), lipase (formation of biofilm and inhibition of host immune response), hyaluronidase (invasion and spreading factor), haemolysins (exotoxins, alpha-, beta- and gamma-haemolysin), leukotoxins, enterotoxins, and exfoliative toxins. Among these, the majority of virulence factors have been confirmed in strains isolated from poultry including antibiotic resistance and toxic shock syndrome toxin-1 (Foster and Höök, 1998; Sato *et al.,* 2000; Hu *et al.,* 2012; Nemati *et al.,* 2013). Still, virulence factors and pathogenesis are not completely elucidated in poultry. Future research with application of various modern methodologies is necessary for understanding of the pathogenicity of staphylococcal strains and development of (opportunistic) infections.

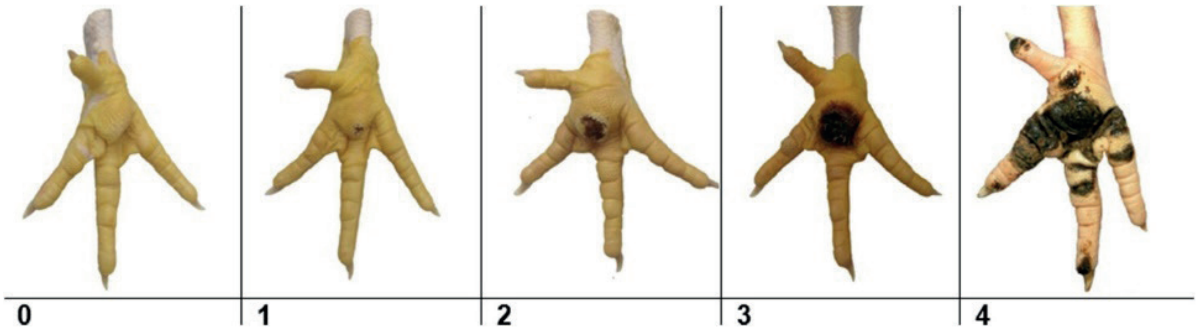
Infections in poultry

Infections are common in poultry and often become systemic involving the bones, joints and tendons. As mentioned earlier, the route of entry and pathogenesis have not yet been well understood, but generally, pathogenic strains can enter the host *via* damaged mucosae and skin (damage to the epithelium and/or mucous membranes by abrasions, trauma, surgical procedures or other injury), open navel of newly hatched chicks, vaccination site, through respiratory tract or after damage caused by other diseases (e.g., chicken infectious anaemia, Marek's disease and other immuno-compromising disorders). Experimentally, infection was caused after intravenous administration of bacteria (10^5CFU/kg body weight) (Mutlib *et al.,* 1983; Zhu and Hester, 2000; Andreasen, 2020).

Within a short incubation period after the pathogen has been introduced into animal deeper tissue (around 2-3 days), the number of heterophils (and their infiltration) increases. Once in the circulation, clinical signs may become visible. These sites of infection can be bones, tendons and joints (tibiotarsal and stifle joints), skin, bursa, yolk sac, vertebrae, eyelids, open navel, heart, liver and lungs (formation of granulomas). *Staphylococci* have affinity for collagen-rich sites (articular surface of joints, synovial layers around joints and tendons) and growth plate of actively growing bones (Mutlib *et al*., 1983; Andreasen *et al*., 1993).

Staphylococcosis is either systemic infection characterised by gangrenous dermatitis and septicaemia or localized infection (pododermatitis, arthritis, tenosynovitis, osteomyelitis, omphalitis). Poor immune status of the host, as in other opportunistic infections, facilitates development of the disease (host organism fails to stop entry of bacteria into deeper tissue). In the acute phase, birds have ruffled feathers, show lameness and fever, followed by the signs of depression and death. In chronic stage, clinical signs on the affected sites are visible, e.g., swollen joints, birds are sitting on their hocks, hardly stand or move, and death occurs due to decreased mobility (inability to access food and water) (Mutlib *et al.*, 1983).

In poultry industry, most common is avian pododermatitis (plantar abscess or 'bumblefoot'). It is a soft tissue (subcutaneous tissue in the feet) infection by *S. aureus* with subsequent septicaemia and/or formation of abscesses. It usually starts after abrasion in food pads and leads to the arthritis-synovitis-osteomyelitis complex, swelling of the footpad and toes, lameness and death (Butterworth, 1999; McNamee *et al*., 2000; Zhu and Hester, 2000) (Figure 3). Sudden death syndrome and severe septicaemia also are often caused by this pathogen. It can occur in laying birds during hot weather, in hatchery, as a secondary infection or in case of gangrenous dermatitis complicated with septicaemia (affected areas of the skin are dark red to blue-green, sharply defined from the surrounding area) (Bisgaard *et al*., 2010; Stepien-Pysniak *et al*., 2017). Omphalitis ('mushy chick' disease) with yolk sac infection occurs in the hatchery. Infection is caused by pathogens present in the breeder flock, environment, or *via* contact of humans (Andreasen, 2020).



Scoring of footpad dermatitis in poultry according to the Welfare Quality Assessment Protocol (Welfare Quality, Citation2009). ©A Butterworth, University of Bristol.

Figure 3. Development of footpad dermatitis and scoring system (from: Kittelsen *et al*., 2024).

Diagnosis

Diagnosis is made according to clinical signs, pathology, and isolation and identification of the pathogen. The best result will be achieved by a combination of phenotypic and genotypic testing. Isolation of the pathogen confirms staphylococcosis and excludes other infections caused by bacteria from the genera *Pasteurella, Salmonella, Mycoplasma, E. coli* bacteria or some viruses. In certain cases, infection is caused by several pathogenic genera.

Treatment and economic significance

Currently, prevention is the best cure since there is no effective vaccine against this pathogen. Good husbandry practice should include a number of preventive measures such as clean and safe environment and prevention of other (immunosuppressive) disorders. Addition of supplements (enzymes, probiotics, extracts) in poultry diets has a potential of improving nutrient digestibility of food, inactivating certain anti-nutritional factors, modulating microflora populations, thus also improving the immune system of animals (Cengiz *et al.*, 2012). Consequently, the birds will have better health and performance and thus be less prone to diseases caused by (opportunistic) pathogens.

Besides unwanted health effects in animals, these pathogens cause significant economic losses in poultry production (chickens and turkeys). The losses are caused by decreased weight gain, decreased egg production, morbidity and mortality (osteomyelitis and septicaemia) and contamination of carcasses at slaughter (green-discoloured livers) (Moon *et al*., 2015).

Additional concerns

Antibiotic resistance is an important problem in veterinary medicine. Spreading of resistance among pathogens is a risk of diseases in animals or as a potential source of transmission to other animals, humans and environment (Weese *et al*., 2010; Moon *et al*., 2015; Kozajda *et al*., 2019). In humans, *Staphylococcus* is considered as a major opportunistic pathogen that can cause life-threatening infections. Infection in humans is mainly acquired through direct contact with infected people, objects and environment. Resistance to antibiotics (penicillin) was early developed and spread in the community *via* acquisition of a plasmid carrying gene (penicillinase). Similarly, the same resistance was developed to methicillin. In poultry, the occurrence of MRSA is increasing at an alarming rate (Lee, 2003; de Boer *et al*., 2009). Additionally, certain pathogenic strains (MRSA strain CC398) that have been reported in animals (pigs, cattle, chicken, horses, and companion animals) might cause infection in humans if they come in contact with live animals (EFSA, 2009).

Conclusion

Staphylococcus species were one of the first discovered pathogens, but they still pose a health risk for humans and animals. Development of new methodologies has enabled new insights into the classification of species and defining their role in infection. Still, a significant amount of data on virulence factors, pathogenesis and treatment of staphylococcal pathogens are missing. In addition, we are currently facing a number of resistant pathogens, including MRSA clones worldwide. Defining the sources and routes of infection can help prevent the spread of resistance and pathogens. Use of preventive measures and alternative approach to upgrade the animal immune status can improve health and prevent spread of virulent bacteria to other animals, humans and environment.

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STAFILOKOKNE INFEKCIJE U PERADI

Sažetak

Rod *Staphylococcus* uključuje važne komenzalne i (oportunistički) patogene bakterije u ljudi i životinja. Većina normalno nastanjuje kožu i mukozu ptica i sisavaca. Stafilokokoza dolazi u obliku sistemske infekcije koju karakteriziraju gangrenozni dermatitis i septikemija ili kao lokalna infekcija (pododermatitis, artritis, tenosinovitis, osteomijelitis, omfalitis). Kao i kod drugih oportunističkih infekcija, loš imuni status domaćina pogoduje nastanku bolesti. Stafilokokoza može uzrokovati ekonomske gubitke u peradarskoj proizvodnji. U sadašnje vrijeme prevencija je najbolji lijek budući da nema učinkovitog cjepiva. Nadalje, antimikrobna rezistencija još uvijek je značajan problem u veterinarskoj medicini, a uključuje i terapiju stafilokokoze. Razvoj novih tehnologija omogućio je nova saznanja o patogenosti i rezistenciji, iako nedostatak ključnih podataka onemogućuje razvoj novih učinkovitih terapija. Stoga održavanje zdravlja u peradarskoj populaciji osigurat će sigurnost u hranidbenom lancu, spriječiti pojavu neželjenih infekcija u ljudi te širenje patogena u okoliš. Daljnja istraživanja neophodna su za definiranje zoonotskog potencijala stafilokoka te pronalaženje alternativnih načina liječenja rezistentnih sojeva.

Ključne riječi: *Staphylococcus*, infekcija, perad, rezistencija, stafilokokoza

Received - primljeno: 05.08.2025.
Accepted - prihvaćeno: 03.10.2025.