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ISOLATION OF PASTEURELLA MULTOCIDA SUBSPEC.
MULTOCIDA FROM CHRONIC PERiapICAL LESION

ABSTRACT: This study presents five isolates of Pasteurella multocida subsp. multocida, isolated from chronic periapical inflammatory lesion. We described the methods of sampling and cultivation as well as diagnostic criteria. Pasteurella multocida was diagnosed on the basis of characteristic cultural and tinctorial properties and the facts that all strains produced indole and induced ornithine decarboxylation, glucose, saccharose and maltole fermentation. Isolates produced neither urease, nor fermented lactose and maltose. Further classification to subspecies multocida was based on the fact that all investigated isolates fermented trehalose, xylose and sorbitol, the traits which are diagnostically significant for the species. Patients deny any contact with farm animals or pets, which indicates a possible aerosol transport and animal-human as well as human-human infection. We consider that this organism should be paid more attention by dentist, oral surgeons and microbiologists.

KEY WORDS: Pasteurella multocida, Periapical Lesion, Izolation

INTRODUCTION

Pasteurella multocida is a commonly isolated animal pathogen (Ballows et al., 1991); however, it is a rarely isolated in humans and its etiology is still unclear. There are reports on isolation from goat respiratory organs, wounds and skin burns (Ho, Bush, 2000). Recently, this rare agent has been implicated in severe systemic illnesses in humans, such as endocarditis (Fukumoto et al., 2002), lung abscess (Hazoard et al., 2000), menin-
gitis (Wade et al., 1999) and septicemia after animal bite (Shimizu et al., 1995). Diseases in humans are commonly attributed to the contact with farm animals (Isenberg and D'Amato, 1990; Shimizu et al., 1995) or pets (Arashima et al., 1992), since the Pasteurella species is recognized as "normal flora" in the oral cavity of cats, dogs and other domestic and wild animals. Human infection can also occur after non-bite or other direct animal exposure; usually by aerosol droplet-route by kissing the pet (Arashima et al., 1992).

All five patients in whom this microorganism was isolated denied any contact with animals, including pets. In the available literature from our country (Popović et al., 1974; SOKOLOVIĆ, 1975) as well as from international sources (Grossman, 1959; Spatafore et al., 1990) we did not find a single references on isolation of Pasteurella species as a causative agent or accidental finding in chronic periapical inflammatory lesion. In that respect, we are of the opinion that our isolation of Pasteurella multocida subspecies multocida from two radicular cysts and three granulomes positioned around the tooth root is the result worth to be presented.

MATERIAL AND METHODS

Sampling and transportation

Specimens were obtained aseptically, in the operation room. The mucosa of the oral cavity was disinfected with 0.5% asepsole and 76% alcohol. After lifting of mucoperiostal lobe, steel trephine was used to remove buccal cortex of the jawbone and to make an opening wide enough to approach the outer wall of the granuloma or a cyst. A sterile syringe was used for aspiration of the lesion-content, which was then transferred to a thyoglycolate broth and submitted to the laboratory. Specimens were examined immediately.

MICROBIOLOGICAL METHODS

Aliquots of thyoglycolate broth were cultured on agar plates containing 10% sheep blood, one nutritive and one McConkey agar, and Sabouraud medium. Both blood and a nutritive agar were cultivated/inoculated simultaneously with and without Staphylococcus aureus growth line. One of each blood plates were incubated under microaerophillic conditions, while the remaining plates were incubated under aerobic conditions at 37°C, by the method described by Suvajdžić (Kapetanov, 2000). The remaining thyoglycolate broth was cultivated at 37°C and subcultured after 48 hours on blood agar plates. Plates (one of each sample) were cultivated under aerobic, microaerophillic and anaerobic conditions. All primarily inoculated plates were examined after 18 hours of incubation. Colonies suspected to be Pasteurella were Gram-stained and subcultured to McConkey agar, blood agar and nutritive agar. Biochemical series included the following: Kligler, liquid indole, urease by Christensen, ornit-
hin, glucose, lactose, saccharose, maltose and mannitol by the method recom-
mended by Quinn (Quinn, 1998).

Further differentiation to subspecies was done on the basis of the ability
to ferment trehalose, xylose, arabinose, sorbitole and dulcitol. Diagnostic test
of susceptibility to penicillin disc was also performed.

RESULTS

After incubation period of 18 hours, visible colonies were observed on
two blood agar plates, cultivated under aerobic and microaerophillic condi-
tions. The colonies appeared bigger in conditions of increased CO₂ tension.
Isolates did not show any dependence on staphylococcal growth.

Colonial grown on the nutritive agar did not require addition of blood
factors for growth.

Colonies were delicate, dew-drop like, grayish, butter-like in consistence,
shiny and convex. They were easily removable and dispersible. Neither a com-
plete nor an incomplete erythrocyte lysis was observed. On a nutritive agar,
colonies of the same traits grew slightly slower and were smaller than the co-
lonies on the blood agar. No growth of primary cultures or subcultures occur-
red on McConkey and Sabouraud media.

Subcultures were performed from the thyoglycolate medium after 48 ho-
urs of incubation. Growth was observed in aerobic and anaerobic conditions,
but the best growth rate was obtained under microaerophillic conditions. Colo-
nies exhibited the same characteristics as the primary isolates. Preparations
were made both from primary isolates and subcultures and subjected to Gram-
staining technique. In Gram stained preparations, after 18 hours, gracile,
short, Gram-negative rods and coccobacils were observed, sizing around 1
micron and showing strong bipolar staining.

All isolates revealed positive reaction for cytochromoxidase and catalase
production.

BIOCHEMICAL CHARACTERISTICS

Two biochemical series were made of each specimen — one from the
primarily cultivated plate and one from the subculture after incubation in
thyoglycolate broth. There were no deviations among the series. All strains
produced indole, decarboxilated ornithin, fermented glucose, saccharose and
mannitol. The investigated isolates showed negative results for urease produc-
tion and for lactose and maltose fermentation.

On the basis of the presented parameters, the strains were defined as Pa-
steurella multocida. Further classification to three possible subspecies determi-
ned the organism as Pasteurella multocida subspecies multocida, due to the
fact that all investigated isolates fermented trehalose, xylose and sorbitol,
which are traits diagnostically significant for the species.
DISCUSSION AND CONCLUSION

Our positive findings in oral cavity, upper and lower parts of respiratory organs (Ho, Bush, 2000) indicate the possibility of isolation of this organism in humans, which can cause a severe disease especially in compromised hosts and under conditions of decreased general or specific immunity (in our patients — the preceding dental problems).

With respect to the cultural, tinctorial and biochemical characteristics (Suvajdžić, 2000), our isolates corresponded with the available literature (Holmes et al., 1995; Quinn, 1998). Since Pasteurella was isolated from five patients with a pronounced pathological process, presenting the only bacterial isolate, we believe it was etiologically significant. We are of the opinion that this microorganism deserves particular attention of dentists (sampling and transportation of specimens) and bacteriologists (since its colonial appearance and routine biochemical examination of isolates may misdirect them towards “non-fermenting microorganisms” which present normal bacterial flora or oral cavity or towards weakly fermenting representatives of the genus Enterobacteriacea).

REFERENCES


IZOLACIJA PASTEURELLA MULTOCIDA IZ HRONIČNIH PERIAPIKALNIH INFLAMATORNIH LEZIJA*

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Резиме

У раду је приказано пет изолата Pasteurella multocida subsp. multocida изолованих из хроничних периапикалних инфламаторних лезија. Описан је начин узимања материјала, бактериолошке обраде узорка и дијагностички критеријум. На основу карактеристичних и интензивних особина, као и чињенице да су сви сојеви продуктовали индол, декарбоксидисали орнитин, ферментисали глукозу, сахарозу и манитол, а ни један испитивани изолат није продуктово урезао, нити ферментисао лактозу и малитозу, постављена је дијагноза Pasteurella multocida. Subspecies multocida одређена је чињеницом да су сви ис-

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питани изолати ферментисали трехалозу, ксилоzu и сорбитол. Ни један од пацијената није био у контакту са фармским животињама нити кућним жељубимцима, што указује на могућност аеросолног транспорта како са животиње на човека тако и са човека на човека. Мишљења смо да овом микроорганизму треба посветити више пажње од стране стоматолога, оралних хирурга и микробиолога.