

# First report of a *Staphylococcus caprae* isolated from middle ear fluid of an infant with recurrent acute otitis media

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## Abstract

*Staphylococcus caprae* was originally isolated from goat milk. This uncommon coagulase-negative staphylococcus, usually associated with animals, has only infrequently been detected in human clinical specimens. Its association with acute otitis media has not been demonstrated so far. The study reports the first isolation of *S. caprae* from the middle ear fluid of a 12-month-old infant with recurrent, bilateral acute otitis media. Biochemical traits and susceptibility pattern of the isolated strain are also presented.

## Key words

*Staphylococcus caprae*, recurrent acute otitis media, middle ear fluid, antimicrobial susceptibility, biochemical profile

## INTRODUCTION

*Staphylococcus caprae* was originally isolated from goat milk by Devriese et al. in 1983 [1]. It is the predominant species among coagulase-negative staphylococci colonizing the skin covering the udders of healthy goats [2]. Since the time of its first isolation, only infrequent cases of human infections caused by *S. caprae* have been reported, among them endocarditis, sepsis, urinary tract infections, bone and joint infections [2–5]. Recently, meningitis following the infection of an intraspinal device and central line-associated bacteraemia have been described [6, 7]. One case of mastoiditis and cases of acute otitis externa have also been documented [8, 9]. Reported human *S. caprae* infections have been acquired nosocomially following the administration of antibiotics [2, 3]. The presented study reports the first isolation of *S. caprae* from the middle ear fluid (MEF) of a 12-month-old infant with recurrent, bilateral acute otitis media (AOM).

## CASE REPORT

The patient was a 12-month-old male who presented to the paediatric emergency room with rhinorrhoea and cough. He was weepy, anxious, agitated, yet afebrile. His parents reported that these symptoms appeared two days before and developed despite symptomatic treatment with paracetamol. In the medical history there were 4–5 AOM episodes during the last five months, diagnosed by general practitioners and treated with antibiotics. The last AOM incident, which occurred about one month before, was treated in the hospital

with intravenous cefotaxime for 5 days. The child did not receive a conjugated pneumococcal vaccine.

On paediatric examination the patient had normal vital signs. Laryngological examination revealed hyperaemia of throat mucosa and nasal concha, as well as abundant sero-mucous secretion in both nasal passages. Otoscopy revealed bilateral AOM. Tympanic membrane of the right ear was congested, matt, entirely bulged, tense; of the left ear: grey-pink, matt, bulged in lower quadrants. Objective hearing examination was performed. Distortion product otoacoustic emission was absent bilaterally. The presence of inflammatory exudate in middle ear was confirmed in impedance audiometry: bilateral type B tympanogram, no reflexes from stapedial muscles. Blood cell count revealed leukocytosis (21,380/mm<sup>3</sup>) with increased values of both: neutrophils (5,460/mm<sup>3</sup>) and lymphocytes (14,490/mm<sup>3</sup>). Other blood parameters were normal.

The boy was admitted to the hospital and classified for emergency operation under general anaesthesia. Antisepsis of the ear canal before the procedure was performed by installing an Octenisept (Schülke&Mayr) for 1 minute. After its removal by suction, myringotomy was carried out, during which a dense, mucopurulent discharge was found bilaterally draining under pressure from middle ear. An MEF sample was collected from each ear with the use of a sterile suction needle inserted into middle ear cavity through an incision in the tympanic membrane. Both samples were placed in sterile Eppendorf tubes, maintained at air temperature, and transported immediately to the laboratory. The remaining amount of exudate was aspirated. Next, ventilation drainage of middle ear was placed bilaterally. Oral amoxicillin (90 mg/kg/day) and anti-inflammatory treatment was started.

Twenty-four hours after the operation the infant was discharged home in a good general and local state, with the recommendation to present at a follow-up visit after 2–3 days in order to assess the local state and, if necessary,

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to correct antibiotic therapy depending on the results of microbiological examination. The patient presented after 2 weeks with no signs of upper respiratory tract infection. According to the parents' report, treatment with amoxicillin was carried out for 8 days. Both tympanic membranes were grey, with vestigial light reflex. Ventilation drainage was permeable, without any pathological discharge. Behavioural hearing tests were performed, no deviation from the normal state was noted.

**Bacteriology findings.** In the right and left MEF samples, respectively, pure cultures of *S. pneumoniae* and *S. caprae* were identified with the use of routine microbiological methods. Antimicrobial susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing recommendations (EUCAST 2012, version 2.0) [10]. *S. pneumoniae* strain was specified as serotype 19F by means of quellung reaction using antisera provided by Statens Serum Institute (Copenhagen, Denmark). Resistance to oxacillin, erythromycin, tetracycline, clindamycin, and trimethoprim/sulfamethoxazole was detected with the use of a disc diffusion method. The strain was classified as penicillin non-susceptible based on benzylpenicillin minimal inhibitory concentration (MIC = 0.75 mg/L) evaluated by using the E-test (AB Biodisk, Sweden). Identification of *S. caprae* and its drug susceptibility were performed by Vitek 2 Compact (bioMérieux) using GP and AST-P580 cards (Bionumber: 010002002661221; 99% probability). Tables 1 and 2, respectively, show the biochemical traits and antimicrobial susceptibility of the isolated strain.

**Table 1.** Biochemical activity of *Staphylococcus caprae* strain isolated from middle ear fluid

Test or characteristic	Result	Test or characteristic	Result
Haemolysis of sheep blood	+	Enzymatic activity of:	
Production of acid from:		Phosphatidylinositol phospholipase C	-
D-amygdalin	-	Arginine dihydrolase	+
D-xylose	-	Beta-galactosidase	-
Cyclodextrin	-	Alpha-glucosidase	-
D-sorbitol	-	Ala-Phe-Pro aryloamidase	-
D-galactose	-	L-aspartate aryloamidase	-
D-ribose	-	Beta-galactopyranosidase	-
L-lactate	+	Alpha-mannosidase	-
Lactose	-	Phosphatase	-
N-acetyl-D-glucosamine	-	Leucine aryloamidase	-
D-maltose	+	L-proline aryloamidase	-
D-mannitol	+	Beta-glucuronidase	-
D-mannose	+	Alpha-galactosidase	+
Methyl-beta-D-glucopyranoside	-	L-pyrrolidonyl-aryloamidase	-
Pullulan	-	Alanine aryloamidase	-
D-raffinose	-	Tyrosine aryloamidase	-
Salicin	-	Urease	-
Saccharose	-	Arginine dihydrolase	-
D-trehalose	+		
Resistance to:		Growth in 6.5% NaCl	+
Polymyxin B	-		
Bacitracin	+		
Novobiocin	-		

- negative reaction; + positive reaction

**Table 2.** Antimicrobial susceptibility of *Staphylococcus caprae* strain isolated from middle ear fluid

Antimicrobial	MIC (mg/L)	Interpretation
Benzylpenicillin	≥0.5	R
Erythromycin	≤0.25	S
Clindamycin	≤0.25	S
Tetracycline	≤1	S
Fosfomycin	≥128	R
Fusidic acid	≤0.5	S
Trimetoprim/sulfamethoxazole	≤10	S
Gentamycin	≤0.5	S
Tobramycin	≤1	S
Levofloxacin	0.25	S
Moxifloxacin	≤0.25	S
Vancomycin	1	S
Teicoplanin	≤0.5	S
Linezolid	1	S
Tigecycline	≤0.12	S
Cefoxitin screen	NEG	MSCNS

R – resistant; S – susceptible; MSCNS – methicillin-sensitive coagulase-negative staphylococci

## DISCUSSION AND CONCLUSIONS

*Streptococcus pneumoniae* remains one of the most important bacterial pathogens in non-complicated as well as recurrent or persistent AOM. In recalcitrant AOM, pneumococcal strains with higher penicillin MICs or multidrug-resistant proved to be more frequently isolated [11, 12]. Indeed, the *S. pneumoniae* 19F strain was isolated in the MEF obtained from the right ear of the presented patient; the isolate turned out to be penicillin non-susceptible and multidrug-resistant. However, in the left MEF sample, the copious growth of *S. caprae* was detected in pure culture. Simultaneous isolation of 2 different bacteria from each MEF sample proved to be an extremely rare event in bilateral AOM [11, 12]. Nonetheless, in one out of 26 recalcitrant bilateral AOM episodes reported by Pumarola et al. [11], pure cultures of prominent otopathogens, namely *S. pneumoniae* and *Haemophilus influenzae*, were yielded from each MEF sample, showing that infection in both ears is not necessarily caused by the same bacterial species. Hence, the isolation of *S. caprae* in pure culture, together with otoscopically-confirmed AOM signs, strongly suggests its etiological role in the left middle ear inflammatory process in the presented patient. Although *S. caprae* association with AOM has not been documented so far, its role as a pathogen was demonstrated and pathogenicity determinants were detected, including the *ica* operon involved in biofilm formation and a gene encoding a fibronectin-binding autolysin [13]. Interestingly, *ica* operon and surface proteins binding fibronectin have been detected in all human and goat *S. caprae* isolates tested [2, 13]. Biofilm and slime production *in vitro* by some human and goat *S. caprae* strains, contributing to their virulence and protection against antibiotic access, have also been documented [2]. Human infections with *S. caprae* proved to be associated with previous administration of antibiotics and hospitalization [2, 14], which revealed their opportunistic

nature. Of note, both risk factors were present in the current case. The isolated strain proved to be resistant only to penicillin and fosfomicin, the latter feature being considered a helpful presumptive test for the identification of *S. caprae* [2, 14]. The susceptibility pattern of the isolate in the current case turned out to be similar to those reported by Vandenesch et al. [14], Roland et al. [9] and Kini et al. [4]. However, meticillin-resistant *S. caprae* strains have also been reported [7, 15]. The isolate in the current case proved to produce acid from mannitol, similar to 100%, 87% and over 90% of the human strains of *S. caprae* reported by Vandenesch et al. [14], Shuttleworth et al. [8] and Kawamura et al. [3], respectively. In contrast to most human *S. caprae* strains described by Vandenesch et al. [14], and similarly to 53% of those reported by Kawamura et al. [3], the isolate in the current case showed beta-hemolysis on sheep blood agar. Urease activity has been detected in 100%, 93% and over 90% of the human *S. caprae* strains reported by Vandenesch et al. [14], Shuttleworth et al. [8], and Kawamura et al. [3], respectively. The strain isolated from the patient in the current case proved to be urease negative.

The underlying microbiology of AOM is known to change over time in response to various selective pressures, including antibiotic consumption [11, 12]. The presented report shows that in patients with recurrent AOM, exposed to several courses of antibiotics and hospitalized, antibiotic pressure not only selects for resistant strains among recognized, but also allows rare opportunistic bacteria, such as *S. caprae*, to invade the middle ear cavity. Moreover, this account underscores the necessity to perform microbiological examination of MEF samples obtained from both ears in cases of refractory bilateral acute otitis media, since inflammatory process in each ear may be caused by different bacterial species.

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