

Fluoroquinolone-Resistant *Alcaligenes faecalis* Related to Chronic Suppurative Otitis Media, Angola

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We found that 20 (10.6%) of 188 patients with chronic suppurative otitis media in Angola were co-colonized with fluoroquinolone-resistant *Alcaligenes faecalis*, commonly found in birds. A likely explanation for our findings was the use of bird feces by residents as a traditional remedy to prevent ear secretions caused by primary ear infection.

Chronic suppurative otitis media (CSOM) is a common condition in developing countries and in original populations such as the Inuit (1). Mainly, young children are reported to have CSOM; 30%–40% of the population are affected in some geographic areas (2). CSOM is associated with hearing loss caused by perforation of the tympanic membrane, which often leads to chronic infection of the middle ear. Overcrowding, poor hygiene, and low nutrition status, in addition to absence of modern healthcare systems, are contributing factors for CSOM (2). CSOM is caused by a polymicrobial infection including gram-negative species, dominated by *Pseudomonas aeruginosa* and *Proteus mirabilis*, in addition to gram-positive bacteria such as enterococci and staphylococci (3).

During studies of ear discharge caused by otitis media, we detected the gram-negative bacillus *Alcaligenes faecalis* in addition to the commonly isolated bacterial species (3). *A. faecalis* may reside in the human microbiome of the gastrointestinal tract but only occasionally causes disease. Most cases occur in immunocompromised hosts, but in rare instances, *A. faecalis* infection has been described in patients who had acute otitis media, peritonitis, and eye or urinary tract infections (4–6). *A. faecalis* is frequently found in bird fecal specimens and can also cause opportunistic infections in these animals (7).

We examined specimens from 188 patients who had ear discharge related to otitis media who attended an outpatient

ear, nose, and throat clinic in Luanda, Angola, during January–December 2016. This study was approved by the Angolan Medical Council, the director of the Josina Machel Hospital, and the Luanda University Medical Faculty. After cleaning the ear canal with 70% ethanol, we collected discharge with a swab. For nasopharyngeal sampling, we inserted a swab into the nostril past the choana and touched the wall of the nasopharynx. We collected ear and nasopharyngeal samples and stored them in skim milk-tryptone-glucose-glycerol (STGG) medium (8) at -70°C (Public Health Laboratory, Luanda, Angola) before transport to the Riesbeck Laboratory (Malmö, Sweden). Clinical specimens were cultured and bacteria analyzed (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/23/10/17-0268-Techapp.pdf>). We found that 20 (10.6%) patients were colonized by *A. faecalis* (Table). Among patients harboring *A. faecalis*, 14 were male (1–47 years of age) and 6 were female (2–23 years of age). *A. faecalis* was growing in polymicrobial communities, and *P. aeruginosa* was the predominant species in 10 (50%) of the patients. *Proteus mirabilis* was the second most common bacterium ($n = 7$), followed by *Klebsiella* spp. and gram-positive cocci. Eight of the patients had otalgia, and the duration of otorrhoea was >12 months in 4 of those patients.

We tested *A. faecalis* isolates against a series of selected antimicrobial drugs by using broth microdilution (online Technical Appendix Table) and found 100% were susceptible to aminoglycoside amikacin, cephalosporins, and colistin. In addition, 90% were susceptible to gentamicin, tobramycin, and piperacillin/tazobactam, and 75% were susceptible to trimethoprim/sulfamethoxazole. By contrast, most isolates were resistant to the fluoroquinolones ciprofloxacin (100%) and levofloxacin (82.6%).

A. faecalis has been described in patients with CSOM (9), but for us, this observation was initially an enigma. We considered the possibility of a contaminant from the endogenous fecal microbiome. We found, however, that to prevent ear discharge, patients occasionally filled their external auditory canals with dove or pigeon feces. Some patients in the geographic area also used cockroach paste, palm oil, sweet olive oil, sewing machine oil, or breast milk to prevent ear discharge. The origin of *A. faecalis* from birds would be a likely explanation for the appearance of this particular bacterial species among this study cohort.

Topical treatment using antimicrobial drugs in combination with keeping the ear canal clean and dry is the mainstay of therapy for CSOM (10). It remains to be confirmed whether *A. faecalis* colonization plays a crucial role for disease progression or merely is a contaminant. However, the microbiological findings in this study should be a note of caution because all *A. faecalis* isolates we obtained were

Table. Co-colonizing bacterial species, clinical characteristics, and demographics of 20 patients colonized with *Alcaligenes faecalis* who had ear discharge related to otitis media, Luanda, Angola, January–December 2016*

Patient no.	Co-colonizing bacterial species	Patient age, y/sex	Otalgia	Otorrhoea type, duration, mo	Neighborhood of residence
1	<i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Proteus</i> spp., <i>Enterococcus faecalis</i> , <i>Enterococcus avium</i>	10/M	No	Unilateral, 1	Coelho, Viana†
2	<i>P. aeruginosa</i> , <i>Proteus mirabilis</i> , <i>Citrobacter freundii</i>	8/F	No	Bilateral, <0.5	Cazenga, Cazenga‡
3	<i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>Morganella morgani</i> , <i>Providencia rettgeri</i> , <i>E. faecalis</i>	20/M	No	Bilateral, >3	Tala-Hady, Cazenga‡
4	<i>P. mirabilis</i> , <i>C. freundii</i> , <i>E. faecalis</i>	8/M	No	Unilateral, >0.5	Hoji-ya-Henda, Cazenga‡
5	<i>P. aeruginosa</i> , <i>Staphylococcus saprophyticus</i>	5/F	No	Unilateral, >12	Bairro 6, Viana‡
6	<i>P. mirabilis</i> , <i>Corynebacterium striatum</i> , <i>Citrobacter amalonaticus</i>	4/M	No	Bilateral, >12	Antonove, Cazenga‡
7	<i>Klebsiella pneumoniae</i> , <i>C. freundii</i>	23/F	Yes	Unilateral, >12	Rangel, Luanda
8	<i>P. aeruginosa</i> , <i>Providencia stuartii</i> , <i>Stenotrophomonas maltophilia</i>	17/M	Yes	Unilateral, >6	Asa Branca, Cazenga‡
9	<i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>Enterobacter cloacae</i> , <i>Arthrobacter</i> spp.	20/F	Yes	Unilateral, <0.5	Asa Branca, Cazenga‡
10	<i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>K. pneumoniae</i> , <i>M. morgani</i>	22/M	Yes	Unilateral, >12	Cazenga, Cazenga‡
11	<i>P. aeruginosa</i> , <i>P. stuartii</i> , <i>Citrobacter koseri</i>	7/F	No	Unilateral, >12	Sambizanga, Luanda
12	No other species‡	13/M	No	Unilateral, >12	Saurimo, Saurimo§
13	<i>M. morgani</i>	5/M	Yes	Unilateral, >12	Pimba, Saurimo§
14	<i>Arthrobacter</i> spp.‡	6/M	No	Bilateral, NA‡	Saurimo, Saurimo§
15	<i>P. aeruginosa</i> , <i>Streptococcus constellatus</i>	14/M	Yes	Unilateral, >12	Saurimo, Saurimo§
16	<i>P. mirabilis</i> , <i>Arthrobacter</i> spp., <i>E. faecalis</i>	1/M	Yes	Unilateral, >3	Viana, Viana‡
17	<i>P. aeruginosa</i> , <i>Klebsiella oxytoca</i>	2/M	Yes	Unilateral, >3	Sanzala, Viana‡
18	<i>Arthrobacter</i> spp.‡	ND/M	Yes	Unilateral, >12	Tshinganja 2, Saurimo§
19	<i>Arthrobacter</i> spp., <i>P. stuartii</i>	47/M	No	Unilateral, >12	Estalagem, Viana‡
20	<i>Enterococcus faecium</i>	2/F	NA	NA, NA	NA¶

**A. faecalis* isolated from external ear discharge except as indicated. NA, not applicable; ND, no data.

†Province Luanda.

‡*A. faecalis* isolated from nasopharyngeal swab.

§Province Lunda Sul.

¶Province Namib.

resistant against the most commonly used fluoroquinolone, ciprofloxacin. An alternative strategy would therefore be to consider colistin as topical treatment or supplement with orally administered amoxicillin/clavulanic acid in the treatment of more severe cases. The supply of topical agents in Luanda is unknown; therefore, the optimal treatment of patients colonized with *A. faecalis* should be determined and appropriate supplies obtained.

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Spread of Chikungunya Virus East/Central/South African Genotype in Northeast Brazil

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We investigated an outbreak of exanthematous illness in Maceió by using molecular surveillance; 76% of samples tested positive for chikungunya virus. Genetic analysis of 23 newly generated genomes identified the East/Central/South African genotype, suggesting that this lineage has persisted since mid-2014 in Brazil and may spread in the Americas and beyond.

Dengue virus (DENV), Zika virus (ZIKV), and chikungunya virus (CHIKV) co-circulate in Brazil, are predominantly transmitted by *Aedes aegypti* mosquitoes, and cause similar clinical symptoms upon infection, complicating epidemiologic surveillance. Brazil harbors the highest diversity of CHIKV in the Americas; both the Asian and the East/Central/South African (ECSA) lineages circulate in the country (1). Despite high prevalence of CHIKV in Brazil (352,773 notified cases during January 2016–May 2017) and its widespread distribution (2), little is known about its transmission. We report a molecular and genomic investigation of an outbreak of CHIKV infection in Maceió, Alagoas state, Northeast Brazil.

During March 30–May 3, 2016, ≈12,000 patients visited 2 private hospitals in Maceió; roughly 70% of them had exanthematous illness symptoms compatible with DENV, CHIKV, or Zika virus infection. We analyzed 273 randomly chosen samples by using molecular diagnostics and virus discovery methods. The study was approved by the Faculty of Medicine from the University of São Paulo Review Board, and we obtained informed consent from all participants.

Analyzed samples were from patients who were on average 37 years of age (range 1–86 years); 175 (64%) were female, and 198 (73%) resided in Maceió municipality. Diagnostic tests for DENV, ZIKV, and CHIKV confirmed that 208 (76%) were positive for CHIKV RNA (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/23/10/17-0307-Techapp1.pdf>). In addition, 66 (24%) were positive for Zika virus RNA and 36 (13.2%) were co-infected with CHIKV and Zika virus, consistent with Zika virus circulation in Northeast Brazil in mid-2016 (3). We detected no DENV infections. Cycle threshold (C_t) values for CHIKV RNA-positive samples were lower (average $C_t = 24.6$) than those for ZIKV (average $C_t = 33.5$).

We applied a metagenomics next-generation sequencing protocol to 38 randomly chosen CHIKV RNA-positive samples (4) (online Technical Appendix). We recovered 23 CHIKV genomes (>4,000 bp) by using the MiSeq Sequencer (Illumina, Inc., San Diego, CA, USA); mean genome coverage was 72× and mean depth coverage 207× (online Technical Appendix Table 1). We also

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Technical Appendix

Sampling of Ear Discharge and Nasopharyngeal Swabs

Culture conditions

Clinical specimens were cultured on hematin agar, Columbia CNA agar (Oxoid), and finally UriSelect agar (Oxoid) supplemented with vancomycin incubated at 35.5°C in 5% CO₂ or in aerobic conditions (UriSelect) for 16–18 h. Species identification was done by Matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF MS).

Antimicrobial susceptibility testing

MICs for *A. faecalis* were interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoints for *Pseudomonas (I)* (Technical Appendix Table). Due to better concordance, we included breakpoints for *Enterobacteriaceae* regarding trimetoprim-sulfamethoxazole. Our observation that most *A. faecalis* isolates were susceptible to gentamicin was in contrast to a previous study showing that *A. faecalis* were resistant against this particular aminoglycoside (2).

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Technical Appendix Table. MIC distributions for *Alcaligenes faecalis* (20 isolates) and interpretation according to tentative ECOFFs, and EUCAST clinical breakpoints for *Pseudomonas*.*†

Antimicrobial agent	MIC (mg/L)											Tentative ECOFFs*		<i>Pseudomonas</i> breakpoints†		
	≤0.125	0.25	0.5	1	2	4	8	16	32	64	≥128	WT	NWT	S	I	R
Piperacillin-tazobactam	–	–	–	14 ‡	3	1	–	1	–	–	1	18	2	19	–	1
Cefepime	–	–	–	–	–	1	16	3	–	–	–	20	0	17	–	3
Ceftazidime	–	–	–	1	12	5	–	1	1	–	–	18	2	18	–	2
Ciprofloxacin	–	–	2	5	5	5	–	3§	–	–	–	17	3	2	–	18
Levofloxacin	–	–	4	8	5	–	–	3§	–	–	–	17	3	12	–	8
Amikacin	–	–	–	–	–	14	6	–	–	–	–	20	0	20	–	–
Gentamicin	–	–	–	–	18	2	–	–	–	–	–	20	0	18	–	2
Tobramycin	–	–	–	–	18	1	1	–	–	–	–	19	1	18	–	2
Colistin	–	–	–	7	13	–	–	–	–	–	–	20	0	20	–	–
Trimethoprim-sulfamethoxazole	14	1	–	–	–	2	1	–	2¶	–	–	15	5	15#	2	3

*Tentative epidemiologic cutoff (ECO) values are based on the MIC distributions in this table. EUCAST, European Committee on Antimicrobial Susceptibility Testing antimicrobial susceptibility testing; Bold text and WT indicate wild type; NWT, non-wild type; S, susceptible; I, intermediate; R, resistant. Dashes indicate nonsusceptibility.

†EUCAST Clinical Breakpoint Tables v 7.0 (1).

‡≤1 mg/L

§≥16 mg/LfMIC

¶≥32 mg/L

#For trimethoprim-sulfamethoxazole, breakpoints for *Enterobacteriaceae* were used.