Rothia Bacteremia
A 12-Year Experience at a Tertiary-Care Teaching Hospital in Szeged, Hungary

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Background: The genus *Rothia* are nonmotile, aerobic or facultative anaerobic, non–spore-forming Gram-positive cocci, which are considered low-grade pathogens with relatively few known virulence determinants.
Methods: During our retrospective, single-center cohort study, microbiological data were collected corresponding to the 12-year period (January 1, 200 to December 31, 2017), at the Institute of Clinical Microbiology, University of Szeged.
Results: A total of 37 individual *Rothia* isolates were identified (3.1 ± 1.9/years; range, 0–6 isolates), 28 were *Rothia dentocariosa*, 9 were *Rothia mucilaginosa*. The affected patients presented with a slight female dominance (21 of 37; female/male ratio, 1:31); the median age of the affected patients was 57 years (range, 1–86 years). In the majority of blood cultures (n = 22), *Rothia* species were the only isolated microorganisms. All of the tested strains were susceptible to benzylpenicillin, vancomycin, ciprofloxacin, moxifloxacin, linezolid, and rifampicin.
Conclusions: *Rothia* species may readily be misidentified as staphylococci, streptococci, or corynebacteria, both the clinical microbiologists and physicians should be aware of the possible etiological role of these microorganisms during their clinical practice, especially if the relevant risk factors are present in these patients.

Key Words: bacteremia, retrospective, *Rothia*, *R. Dentocariosa*, *R. mucilaginosa*, epidemiology

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The genus *Rothia* (first designated by Georg and Brown in 1967) is a member of the *Micrococcaeae* family, which includes other genera, such as *Arthrobacter, Dermacoccus, Kocuria, Kytococcus, Micrococcus* (which includes the species *M. luteus*), *Nesterenkonia*, and *Pediococcus*.[1] *Rothia* species (currently T1 encompassing 8 distinct species; see Table 1.) have undergone several taxonomical changes in the last 3 decades, for example, *R. dentocariosa* (the type species of the genus) was previously known as *Nocardia salivae*, *Staphylococcus salivarius*, *M. mucilaginosus,* and *Stomatococcus mucilaginosus*.[2–5] These microorganisms are nonmotile, aerobic or facultative anaerobic, non–spore-forming Gram-positive cocci, which are normal constituents of the flora of the human skin, oral cavity, oropharynx, and upper respiratory tract.[6,7] *Rothia* spp. are considered low-grade pathogens with relatively few known virulence determinants; therefore, these species are rarely significant pathogens in the context of immunocompetent individuals, they are mainly considered as contaminants in relevant cultures.[8] Until recently, the clinical role of *Rothia* species was mainly associated with periodontitis, pericoronitis, and dental caries, in association with other well-known periodontopathogens (such as *Aggregatibacter actinomycetemcomitans, Eikenella corrodens, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola,* etc.)[9]; in fact, *R. mucilaginosa* and/or *R. denticola* are found in the throat cultures around 30% of healthy individuals.[10] However, the increasing role of *Rothia* species as opportunistic pathogens has been noted by several publications, corresponding with the significant rise in the number of immunocompromised patients and invasive surgical interventions worldwide.[7,11,12] The first case of invasive infection (ie, endocarditis) caused by *Rothia* species (namely, *R. dentocariosa*) was published in 1978.[7] Although infective endocarditis is still the most prevalent type of invasive infection,[13] other clinical syndromes associated with these pathogens, such as bacteremia,[6,14] peritonitis,[15] meningitis,[16] pneumonia,[17] biliary tract infections,[6] skin and soft tissue infections,[18] necrotizing fasciitis,[6] bone and joint infections,[19] and endophtalmitis[20] have also been described.

Available literature is limited on the epidemiology of *Rothia* species in invasive infections; therefore, the significance of the isolation of *Rothia* spp. from blood cultures is a controversial topic, especially in case of the polymicrobial infections or if only a single set of blood cultures was available.[7] To make matters more complicated, patients having periodontal lesions or ones that have undergone dental surgery may present with transient *Rothia* bacteremia, which usually clears without medical intervention, if the patient has no underlying conditions affecting the immune system.[21] Most cases of invasive *Rothia* infection (according to the literature) showed a high mortality rate or sequelae (eg, abscess or fistula formation, abdominal aneurisms, peritonitis, vertebral osteomyelitis, cerebral hemorrhages), the relevance of this bacterium in blood cultures should be carefully considered in light of the patient's medical history.[6,22,23] To date, no epidemiological study addressed the topic of invasive *Rothia* infections in Hungary, therefore, the aim of our study was to describe the prevalence and antimicrobial susceptibility of *Rothia* isolates from bloodstream infections and to evaluate the demographic characteristics of these infections at our institution over a 12-year surveillance period.

MATERIALS AND METHODS

Study Design and Data Collection

During our retrospective, single-center cohort study, microbiological data were collected corresponding to the 12-year period between January 1, 2006, and December 31, 2017, at the Institute of Clinical Microbiology, University of Szeged. The Department of Bacteriology in the Institute serves as the primary bacteriological diagnostic laboratory of the tertiary-care teaching hospital (Albert Szent-Györgyi Clinical Center; Szeged, Hungary) in the region; this health care center is responsible for the primary- and specialized care of an estimated population of over 400,000

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TABLE 1. Members of the *Rothia* Genus and Their Relevance in Human Infections[1–20]

Species	Date of Taxonomic Description	Site of First Isolation	Relevance in Human Infections
R. aeria	2004	Patient with endocarditis	+ (0–5%)
R. aerolata	2016	Patient with endocarditis	+ (0–5%)
R. amarae	2002	Sludge of a water sewer	Ø
R. dentocariosa	1967	Patient with dental plaques	+ (25–60%)
R. endophytica	2013	Roots of *Dysophylla stellata* (Lour.)	Ø
R. mucilaginosa	1982	Patient with endocarditis	+ (10–50%)
R. nasimurium	2000	Nasal discharge of a mouse	Ø
R. terrae	2008	Soil (in Taiwan)	Ø

people.[24,25] Data collection was carried out manually by an electronic search of the records in the laboratory information system of the institute, regarding blood culture samples positive for *Rothia* spp. The starting date of data collection has been set from the year 2006, because the electronic laboratory information system was operational since that year. During the study, only the first isolation of the bacteria per patient was included in the analysis; however, isolates with different antibiotic susceptibilities from the same patient were considered as different individual isolates.[26] Time-to-positivity data corresponding to the positive blood culture bottles was also collected.[27] Polymicrobial bacteremia was defined by the isolation of more than one organism in a single blood culture.[27]

Anonymized patient data were also collected on patients who had at least 1 positive aerobic blood culture for *Rothia* spp., which was limited to sex, age at sample submission, and indication for the submission of the blood culture samples.[28] The study was deemed exempt from ethics review by the institutional review board, and informed consent was not required because data anonymity was maintained.

Sample Processing and Identification of *Rothia* Isolates

The processing of blood culture samples arriving to the Institute of Clinical Microbiology was carried out according to current national and international guidelines.[27,29] Between 2006 and 2012, the BD Bactec (Becton Dickinson, Franklin Lakes, NJ) blood culture detection system was used for the incubation of blood culture bottles, whereas between 2013 and 2017, the BacT/ALERT 3D (bioMérieux, Marcy-l'Étoile, France) detection system was used. Blood culture bottles were incubated for 5 days (21 days, if endocarditis was suspected) in both of the abovementioned detection systems.[27,29] If the relevant pathogens presented in significant colony count, the plates were passed on for further processing.

Between 2006 and 2012, presumptive phenotypic (biochemical reaction-based) methods, namely, the API Coryne V2.0 kit (bioMérieux, Marcy-l'Étoile, France; critical enzymatic reactions on the test strips were α-glucosidase and pyrazinamidase positivity and alkaline-phosphatase and β-glucosidase negativity), and the VITEK 2 Compact ID (bioMérieux, Marcy-l'Étoile, France) were used for bacterial identification. After 2013, the abovementioned methods were complemented with the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Bacterial cells from colonies on culture plates were transferred to a stainless-steel target. After drying at ambient temperature, the cells were covered with 1 μL matrix (α-cyano-4-hydroxy cinnamic acid in 50% acetonitrile/2.5% trifluoro-acetic acid) before measurements. Mass spectrometry was performed by the microFlex MALDI Biotyper (Bruker Daltonics, Bremen, Germany), the technical details of the measurements were described previously.[27] The MALDI Biotyper RTC 3.1 software (Bruker Daltonics, Bremen, Germany) and the MALDI Biotyper Library 3.1 were used for spectrum analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method (Liofilchem, Abruzzo, Italy) on Mueller-Hinton agar plates, supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F). The interpretation of the results was based on EUCAST breakpoints for *Corynebacterium* spp.[7] The following antibiotics were tested: benzylpenicillin (indicative of susceptibility for all other β-lactam antibiotics), ciprofloxacin, moxifloxacin, vancomycin, erythromycin, clindamycin, tetracycline, linezolid, and rifampicin.[7,14] During data analysis, intermediate-susceptible results were grouped with and reported as resistant. *Streptococcus pneumoniae* ATCC 49619 (MH-F), *Staphylococcus aureus* ATCC 29213 (MH), *Enterococcus faecalis* ATCC 29212 (MH), *Proteus mirabilis* ATCC 35659 (MH), *Escherichia coli* ATCC 25922 (MH), *Klebsiella pneumoniae* ATCC 700603 (MH), and *Pseudomonas aeruginosa* ATCC 27853 (MH) were used as quality control strains.

Statistical Analysis

Descriptive statistical analysis (including means or medians with ranges and percentages to characterize data) was performed using Microsoft Excel 2013 (Redmond, WA, Microsoft Corp.). Additional statistical analyses were performed with SPSS software version 24.0 (IBM SPSS Statistics for Windows 24.0, IBM Corp. Armonk, NY), using the χ^2 test and 2-sample test. P values less than 0.05 were considered statistically significant.

RESULTS

Demographic Characteristics, Isolation Frequency

During the 12-year study period, a total of 37 individual *Rothia* isolates were identified (3.1 ± 1.9/year; range, 0–6 isolates; highest in 2013 and 2014 [n = 6], lowest in 2007 and 2012 [n = 0]) from blood culture samples. The number of isolates in the first part of the surveillance period (2006–2009) was 6, whereas in the second (2010–2013) and third (2014–2017) part of the study period were 17 and 14, respectively ($P = 0.015$). All (n = 37) isolates were from blood culture samples originating from inpatient departments. The affected patients presented with a slight female dominance (21 of 37; female/male ratio, 1:31); the age distribution of patients was the following: 0–5 years, n = 2; 6–35 years, n = 3; 36–59 years, n = 18; and 60 years or older, n = 13. Overall,

the median age of the affected patients was 57 years (range, 1–86 years); there was no significant difference among men and women ($P = 0.97$).

Nineteen of the 37 isolates originated from the intensive care units of different profiles (cardiology-hematology, surgery, and traumatology), 9 of 37 from the Department of Internal Medicine, 7 of 37 from the Department of Surgery, and 2 of 37 from the Department of Pediatrics. Indications for blood culture sample submission, associated with *Rothia* spp. bacteremia included cardiovascular illnesses (15 of 37), hematological malignancies (10/37) and solid tumors (5/37), recent trauma (4/37) and pneumonia (3/37). No submission corresponding to dental procedures was noted during the surveillance period. Time-to-positivity on the corresponding blood cultures showed the following distribution: 0 to 24 hours in 3 cases, 25 to 48 hours in 6 cases, 49 to 72 hours in 11 cases, 73 to 96 hours in 6 cases, 97 to 120 hours in 7 cases, and over 120 hours in 4 cases.

Species Distribution and Susceptibility of *Rothia* Isolates

Out of the 37 isolates, 28 were *R. dentocariosa*, whereas the remaining 9 were *R. mucilaginosa*. In the majority of blood cultures (n = 22), *Rothia* species were the only isolated microorganism, whereas in 15 samples, additional species were also isolated: CoNS (*S. epidermidis*, *S. haemolyticus*, and *S. hominis;* n = 10); *Escherichia coli,* 3; *S. aureus*, 1; and *Pseudomonas aeruginosa*, 1.

All of the tested strains were susceptible to benzylpenicillin, vancomycin, ciprofloxacin, moxifloxacin, linezolid, and rifampicin (37/37), whereas 2 of 37 isolates were resistant to tetracyclines, whereas 14 of 37 and 16 of 37 isolates were resistant to erythromycin and clindamycin, respectively. No temporal trends or statistically significant differences in resistance were observed during the various parts of the study period ($P > 0.05$).

DISCUSSION

Our study aims to report on the prevalence of *Rothia* species in bacteremia infections at a tertiary-care hospital in Hungary in a 12-year study period (2006–2017). Based on the results of the study in our settings, on average, 3 cases of *Rothia* may be expected per year, predominantly corresponding to patients older than 50 years, presenting with an acute illness or some predisposing factors or immunosuppression. *Rothia* species are considered infrequent etiological agents, which mainly affect immunocompromised patients (therefore, they may be considered as opportunistic pathogens); however, the reports of infections caused by these bacteria in immunocompetent individuals showed a sharp increase since the 2000s, probably due to the significant advancements in the accuracy of diagnostic modalities in clinical microbiology laboratories.[7,30,31] This was highlighted in our study, where much more strains of Rothia were found after 2010, which could be related to the introduction of MALDI-TOF MS–based identification in the laboratory. The list of known risk factors for immunocompetent and immunocompromised individuals for invasive *Rothia* infections is presented in Table 2. Among invasive infections, endocarditis has been the most frequently reported; however, other organ systems (meningitis, pneumonia, peritonitis, skin, and soft tissue infections) may also be affected, especially if the presence of an underlying illness centers around a specific organ (eg, lung cancer and *Rothia* pneumonitis; in these cases, diagnosis was reached from cultures of the pleural fluid and bronchoalveolar lavage).[6–20]

Several case reports or case series in a single institution have been published in the literature[7,12,32–35]; however, comprehensive epidemiological studies on *Rothia* bacteremia are lacking. In a 10-year study at the Mayo Clinic (in Rochester, MI) between 2002 and 2011, 67 adult patients had positive blood cultures for *Rothia* (6.7/year), of which 37.3% presented with symptoms of septicemia and 28.3% had neutropenia and/or some sort of hematological malignancy.[7] As a part of another study in the United States, spanning over 8 years (2006–2014), 29 patients (3.6/year) with a median age of 58 years were detected; in this study, the use of fluoroquinolones and the presence of intravascular catheters during bacteremia was highlighted.[32] Our results show similarities in both the quantitative aspects (ie, the number of cases/year) and qualitative aspects (characteristics of locally affected patients) of the abovementioned 2 reports.

Phenotypically, colonies of *Rothia* species may look similar to *Corynebacterium* spp. and coagulase-negative *Staphylococcus* (CoNS) species; from a clinical standpoint, the differentiation among these pathogens is important, as there are major differences in their virulence, prognosis of infection, and therapy.[1–7,36] Another important distinction is between *Rothia* spp. and the taxonomically close *Pediococcus* spp., as the latter are intrinsically resistant to vancomycin.[37] Colonies are predominantly nonhemolytic on blood agar plates, their consistency (sticky or mucoid colonies) varies among the different members of the genus.[1–7,36] In native wet-mount stains or Gram stains, these microorganisms present as pair or groups of cocci; however, they may present as cocco-bacilli or form filamentous branches as well. When it comes to biochemical/metabolic activity, *Rothia* species are oxidase-negative, catalase-variable, and predominantly positive for glucose, fructose, maltose, and sucrose fermentation (but negative for lactose, mannitol, and xylose, which is an important diagnostic landmark), nitrate reduction, esculin, and gelatin hydrolysis.[1–7,36] They are susceptible to bacitracin and are unable to grow on culture media containing 5% NaCl (cf. staphylococci). Although the pathogenic role of *Rothia* species is infrequent (however, they are common culture contaminants, similar to other members of

TABLE 2. Known Risk Factors for Invasive *Rothia* Infections in Immunocompetent and Immunocompromised Individuals[1–20,30–35]

Immunocompetent	Immunocompromised
Intravenous drug abuse	Severe neutropenia
Cardiac valve diseases	Corticosteroid therapy
Presence of prosthetic devices (eg, heart valves)*	Immunological therapy (eg, TNF-α inhibitors)
Presence of IVCs or CVCs*	Prior use of broad-spectrum antibiotics (eg, fluoroquinolones)
	Hematological malignancies
	Solid tumors
	HIV infection
	Diabetes mellitus
	Chronic liver disease
	Long-term alcohol abuse
	CAPD
	Extensive surgical/dental procedures
	Poor dental hygiene
	Mucositis

* May be a risk factor in both patient groups.

CAPD, continuous ambulatory peritoneal dialysis; HIV, human immunodeficiency virus; IVC, intravascular catheter; CVC, central venous catheter.

the *Micrococcaceae*), they are predominantly found in the databases of commercial biochemical reaction-based identification kits for Gram-positive organisms, allowing for reliable (genus-level) identification.[7] For optimal species-level differentiation among *Rothia* spp., the use of molecular methods (eg, polymerase-chain reaction, 16S rRNA sequencing) or MALDI-TOF MS is usually required. In laboratories without the adequate facilities, *Rothia* species may be misidentified; therefore, their clinical relevance in human infections may also be underreported.[38] As with to other genera that were previously considered as rare pathogens, increase in the interest toward *Rothia* spp. in the literature will presumably increase.[26]

For the empiric therapy of these infections, ceftriaxone, imipenem/cilastatin or meropenem, vancomycin, or the newer fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin) are used.[7] Based on the reports available in the literature, most (>99%) of *Rothia* isolates are susceptible to penicillin G and V, aminopenicillins (ampicillin, amoxicillin), first- to third-generation cephalosporins, carbapenems, and vancomycin; the susceptibility to fluoroquinolones (ciprofloxacin and newer agents) is similarly high, estimated to be around 90% to 95%.[4,6,7,10–20,22,23,30–36] The ratio of tetracycline (due to mutations in the tet efflux pumps), and macrolide-lincosamide-streptogramine resistance (due to several possible mutations in the targets of the respective antibiotics) is reported to be higher, 0% to 30% and 15% to 75%, respectively.[4,6,7,10–20,22,23,30–35,38–40] For this reason, the use of these agents is not recommended as first-line drugs, β-lactam antibiotics represent safer and more efficacious alternatives.[7] In case of tetracycline resistance, the use of doxycycline, minocycline, and tigecycline may still be appropriate (however, tigecycline is not suitable for bacteremia, as it reaches low serum levels).[41]

The following limitations of the present study should be highlighted: (i) the retrospective study design; (ii) identification of bacterial isolates from clinical samples has changed once (in 2013) during the study period; (iii) anamnestic data, laboratory findings (eg, neutropenia, fever) were unavailable, therefore, correlation between the presence of bacteremia and symptoms could not be established (or their possible roles as contaminants); (iv) antibiotic resistance was characterized by the disk diffusion method only, the underlying genetic mechanisms were not further studied[26]; (v) as the amount of isolates studied in this report is limited, the main strength of the present study is providing a brief epidemiological snapshot, together with a very detailed review of the findings available in the literature.

CONCLUSIONS

To summarize our results, *Rothia* species are infrequently isolated Gram-positive organisms, which are increasingly recognized as emerging opportunistic pathogens in immunocompromised patients, and also in immunocompetent individuals, proven by their increasing occurrence found in the literature. As *Rothia* species may readily be misidentified as staphylococci, streptococci, or corynebacteria, both the clinical microbiologists and physicians should be aware of the possible etiological role of these microorganisms during their clinical practice, especially if the relevant risk factors are present in these patients. According to our findings and results in the literature, β-lactam antibiotics may be considered as safe and appropriate choices for therapy, taking into account that the emergence of resistant mutants is a possibility.

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