Original article

Biochemical properties and antimicrobial susceptibility pattern of *Rothia mucilaginosa* isolated from patients with respiratory tract infections

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Abstract:

Background: *Rothia mucilaginosa* is appearing as an alarming pathogen for respiratory tract infection, infection of prosthetic devices and endocarditis. Over the past few years, *Rothia* sp. are frequently identified in a tertiary hospital of northern Bangladesh. Biochemical properties and antibiotic susceptibility pattern of the isolates were determined. ***Methods***: A total 22 isolates of *R. mucilaginosa* were studied to observe the fermentation status of carbohydrate and protein substrates for identification by using BD Phoenix M50 system. Antibiotic susceptibility was tested by disk diffusion method. ***Results:*** All isolates of *R. mucilaginosa* showed variable reaction to almost 50% of the carbohydrate and around 50% of protein- amino acid substrates used. Amikacin, meropenem, imipenem and moxifloxacin showed maximum sensitivity in vitro. ***Conclusion:*** Biochemical characteristics of *R. mucilaginosa* can reveal the vital information necessary for accurately identifying the organism within a sample. This depends on production of various enzymes by the organism. Antibiotic susceptibility test is essential for proper management of patient.

Keywords: *Rothia mucilaginosa*; biochemical property; antibiotic susceptibility; respiratory tract infections.

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Introduction:

Rothia mucilaginosa is a Gram-positive, coagulase-negative, encapsulated, non-spore-forming and non-motile dot shaped bacteria which is a part of the normal oropharyngeal flora [1]. It belongs to the family Micrococcaceae. It is an opportunistic bacteria that causes infections in immunocompromised patients [2]. Cases are also reported in immunocompetent patients [3]. *Rothia* species cause a wide spectrum of diseases including upper and lower respiratory tract infections, bacteremia, endocarditis, catheter-associated bloodstream infection, central nervous system infections, endophthalmitis, spondylodiscitis, osteomyelitis, prosthetic joint infection, cholangitis and peritonitis [4]. Now-a-days, this organism is drawing attention for its frequent isolation from clinical cases [2,3,4].

Biochemical reactions are important parameter for isolation and identification of *Rothia* and for their speciating. Different species of *Rothia* utilizes carbohydrates, protein, amino acid and other substrates by oxidation, fermentation, degradation and hydrolysis differently [5].

Identification of *Rothia* sp. accurately by conventional methods is troublesome. The BD Phoenix™ M50 Automated Microbiology System (Becton,

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Dickinson and Co.; Spark, MD 21152 USA) was used for identification (ID) of *R. mucilaginosa* which provides a better accuracy.

This system utilizes a series of conventional, chromogenic, and fluorogenic biochemical tests to determine the identification of the organism. Enzymatic substrates were used to observe various reactions within the genera of bacteria which is based on the growth of the organisms. Biochemical reactions were implicated by utilization and fermentation of substrates which were detected by special indicator system. If any organism utilized carbohydrate and had produced acid that was demonstrated by change of colour of phenol red indicator. Production of yellow colour was due to enzymatic hydrolysis of either p-Nitrophenyl or p-Nitroanilide compounds. Fluorescent coumarin was released due to enzymatic hydrolysis of fluorogenic substrates. At the same time reduction of resazurin-based indicator was detected mechanically due to carbon utilization by the organism. In addition, there are other tests that detect the ability of an organism to hydrolyze, degrade, reduce, or otherwise utilize a substrate (manufacturer's instruction)

Antimicrobial sensitivity was performed by Kirby-Bauer disc diffusion method [6] in our laboratory to compare the sensitivity pattern with other studies. *Rothia* species are usually susceptible to penicillin, ceftriaxone, meropenem and vancomycin[7]. Though in our studies all strains were resistant to penicillin.

Accurate identification and antimicrobial susceptibility pattern of the isolates tested in this study may contribute to a better management of the infection caused by *Rothia* sp.

Materials and methods:

This cross-sectional descriptive study was carried out at Laboratory Services Department (Microbiology) of Khwaja Yunus Ali Medical College Hospital, Sirajganj, Bangladesh during the winter season, over a period of 6 months from October 2021 to March 2022.

Specimens

Throat swab and sputum were collected from suspected clinical cases of upper and lower respiratory tract infections respectively. A total number of 13 throat swab and 9 sputum samples were collected from the patients as shown in table 1.

Table 1: Clinical specimens and sources

Clinical presentation	Specimens	
	Sputum (n)	Throat swab (n)
Sore throat	-	10
Tonsillitis	-	3
Pneumonia	3	-
Bronchial asthma	3	-
COPD	2	-
Pulmonary tuberculosis	1	-

Inoculation on MacConkey and 5% sheep blood agar plates:

The specimens were inoculated on these 2 media and pure culture were obtained.

Gram staining procedure:

Single colonies were stained by Gram's method and Gram-positive cocci were selected for further biochemical tests in the BD Phoenix™ M50 Automated Microbiology System.

Biochemical tests:

Gram positive, catalase positive with oxidase negative strains were selected for the ID panel of BD Phoenix M50 Automated Microbiology System for biochemical characterization and identification. 4.475 ml of ID broth having bacterial concentration of McFarland standard 0.5 was added to the ID side of Gram-positive panel for each sample (manufacturer's manual). A total 40 substrates including carbohydrates, amino acids, proteins and its derivatives were used for the observation of reactions to identify bacteria. All identification procedures were performed according to the instruction of manufacturer.

Antimicrobial susceptibility test:

Rothia mucilaginosa is not included in the Phoenix AST taxonomy for antibiotic susceptibility test; so an alternative Kirby-Bauer disc diffusion method was performed to investigate the antimicrobial sensitivity [6].

Results:

Gram-stained slide of the isolates confirmed gram positive cocci which is shown in Fig 1. Whitish, pale, non-hemolytic colonies were observed on blood agar medium (Fig 2.).

Reaction to Carbohydrate

A total 20 carbohydrate substrates were used for the identification of the isolates. Of these, only one substrate showed a positive homogenous reaction

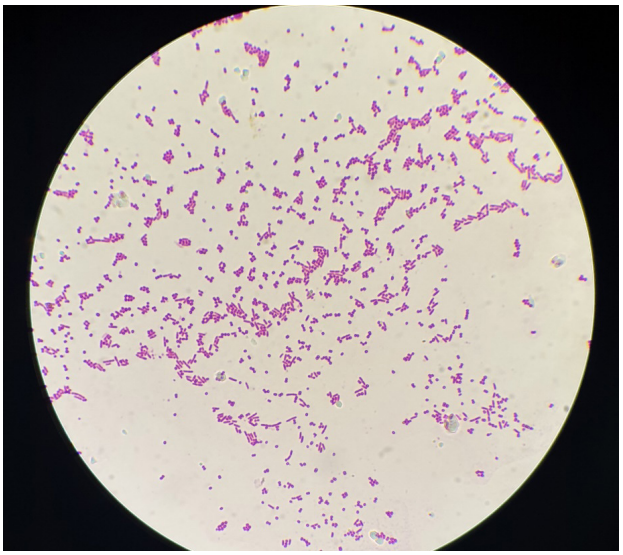

Fig 1: Gram-stained slide of *Rothia mucilaginosa*

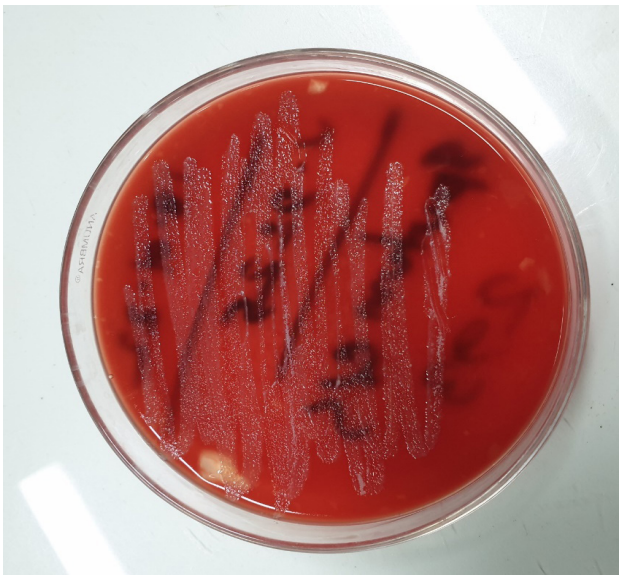

Fig 2: *Rothia mucilaginosa* colonies on 5% sheep blood agar

and 8 substrates showed negative reactions by the organisms. Eleven other substrates showed slight variable reaction by the organisms. The reactions are shown in Table 2.

Reaction to protein and amino acids

All 22 isolates showed a homogenous positive reaction to 7 substrates and all of them showed a negative reaction to only 2 substrates. All isolates produced a variable reaction to remaining 11 substrates. These reactions are displayed in Table 3.

Table 2. Reactions of *Rothia mucilaginosa* to carbohydrates

Carbohydrate substrates	Reactions	
	Positive	Negative
4MU-BD-Cellobioside	1	21
4MU-BD-Glucoside	22	0
4MU-AD-Glucoside	5	17
4MU-BD-Glucoronide	0	22
4MU-N-Acetyl-BD-Glucosaminide	4	18
4MU-BD-Galactoside	5	17
D-Gluconid acid	0	22
3-Methyl Glutaric Acid	0	22
D-Fructose	6	16
D-Mannitol	0	22
BETA-Gentibiose	0	22
D-Sucrose	18	4
Maltotriose	13	9
N-Acetyl-Glucosamine	2	20
D-Trehalose	6	16
D-Tagatose	0	22
Maltose	18	4
Dextrose	16	6
Methyl-α-D-Glucoside	0	22
Esculin	0	22

Table 3. Protein and Amino acid substrates and reactions by *Rothia mucilaginosa*

Protein and amino acid substrates	Reaction	
	Positive	Negative
L-Alanine	22	0
L-Proline AMC	22	0
L-Pyroglutamic acid	9	13

Protein and amino acid substrates	Reaction	
	Positive	Negative
L-Phenylalanine	22	0
L-Tryptophan	12	10
Methionine	21	1
Arginine-arginine	18	4
Glycine-proline	22	0
L-Leucine	22	0
L-Arginine	22	0
L-Histidine	0	22
L-Isoleucine	1	21
Iminodiacetic acid	6	16
Alpha-Ketoglutaric acid	11	11
Thymidine	0	22
Alanine-alanine	5	17
L-Proline PNA	22	0
Valine-alanine	5	17
Phosphate	5	17
Urea	1	21

Antimicrobial susceptibility

A total 29 antibiotics were used to see the susceptibility pattern. Among these, amikacin, gentamicin, meropenem, imipenem, amoxyclav, amoxycillin and moxifloxacin showed the maximum effectivity. Conventional antibiotics like azithromycin, ceftriaxone, ciprofloxacin, levofloxacin, linezolid and rifampicin were less sensitive. All isolates were resistant to penicillin, cloxacillin and cefoxitin. Antimicrobial susceptibility patterns are shown in Table 4.

Table 4. Antimicrobial sensitivity of *Rothia mucilaginosa*

Antibiotics	Sensitivity
Amikacin	86.36%
Azithromycin	50%
Ceftriaxone	63.64%
Cefuroxime	60%
Ciprofloxacin	50%
Gentamicin	77.27%
Levofloxacin	59.09%
Tobramycin	66.67%
Meropenem	90.91%
Imipenem	90.48%
Colistin	40%
Clindamycin	26.31%
Amoxyclav	78.95%
Piperacillin	54.54%
Cloxacillin	0
Penicillin	0
Amoxycillin	77.27%
Ampicillin	61.54%
Ceftazidime	52.38%
Cefixim	28.57%
Cephradine	31.82%
Doxycycline	45.45%
Vancomycin	27.27%
Oxacillin	20%
Moxifloxacin	100%
Linezolid	63.64%
Teicoplanin	54.54%
Rifampicin	58.33%
Cefoxitin	0

Discussion:

R. mucilaginosa (formerly known as *Stomatococcus mucilaginosa*) is a Gram-positive aerobic cocci which is available as normal flora in oral cavity and dental plaques. Recently this organism has been identified as a primary pathogen in many clinical conditions such as pneumonia, meningitis, bone and joint infections, prosthetic device infections, endocarditis with underlying immunocompromised status of host [8].

The pathogenesis and role of this organism as a primary pathogen is not clearly understood till now. One of the phenomena of pathogenicity could be due to the organism's ability to produce a biofilm, similar to other Gram-positive bacteria [9]. This biofilm may be associated with local damage, such as disruption of prosthetic heart valves or loosening of implanted devices, or systemic manifestations, such as septic emboli. Disruption of biofilm is an important component for the management of infection. As the prosthetic devices are coated by biofilm, antibiotic therapy alone is usually ineffective without surgical removal of the device [10,11].

Biochemical properties of this organism is a golden standard for identification. Biochemical reaction to carbohydrate and protein may vary according to epidemiological strains isolated. In our study the reaction to carbohydrate substrates was shown in Table 2. All strains showed a positive reaction to 4

MU-BD-glucoside. All organisms showed negative reaction to 4MU-BD-glucoronide, D-glucoronic acid, 3-Methyl glutaric acid, D-mannitol and Beta-gentibiose substrates. Sucrose, maltose and trehalose showed a variable reaction in our study. On contrary, M. D. Collins et.al observed a positive reaction to all these substrates[5]. About 5% organisms produced urease which differs from another study, where none of the strains were able to produce this enzyme[5].

Reaction to protein and amino acid is an important key for identification. All strains showed positive reaction to 7 substrates namely L-alanine, L-proline-AMC, L-Phenylalanine, Glycine-Proline, L-Leucine, L-Arginine, L-Proline-PNA which may vary from the study of M. D. Collins and Poornima Ramanan et al [5,7]. All strains showed negative reaction to L-histidine and thymidine. With the current setting of our equipment (BD Phoenix™ M50 Automated bacteriological system) three substrates remained unused.

Most of the strains were sensitive to amikacin, meropenem, imipenem and moxifloxacin. These findings can correlate with the study of T G Ramaya et. al and F Michels et. al [12,13]. About 50% of the pathogens were sensitive to conventionally used antibiotics likely azithromycin, ciprofloxacin, ceftazidime, doxycycline and rifampicin. As the equipment could not produce antibiotic sensitivity test due to mechanical lacking, disk diffusion method [6] was used to identify the susceptible antibiotics. Cloxacillin, penicillin, oxacillin and cefoxitin were not effective at all against these strains.

The organism is generally susceptible to penicillin, ampicillin, cefotaxime, imipenem, rifampin, and vancomycin. It is frequently resistant to clindamycin and aminoglycosides, as well as to trimethoprim-sulfamethoxazole and ciprofloxacin [14]. Daptomycin has *in vitro* activity against this organism [15]. Partial resistance to penicillin has been reported in literatures[13,16]. Therefore, vancomycin is recommended as empirical therapy while awaiting susceptibility testing. However, the administration of amikacin, imipenem, meropenem and moxifloxacin can be a solution for the infection caused by *R. mucilaginosa*.

This study reveals a critical decision for the identification of organism as well as the selection of an effective antibiotic. Further study is needed to establish the role of *Rothia* sp. as a primary pathogen. Tissue culture, animal inoculation, cytotoxicity and molecular tests are required for early identification and to establish the role of *R. mucilaginosa* as a primary pathogen.

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