View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Open Access Macedonian Journal of Medical Sciences

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1926-1930.
https://doi.org/10.3889/oamjms.2019.551
eISSN: 1857-9655
Basic Science

Sputum Quality Assessment Regarding Sputum Culture for Diagnosing Lower Respiratory Tract Infections in Children

Gorica Popova[1*], Katerina Boskovska[2], Ivana Arnaudova-Danevska[2], Olga Smilevska-Spasova[2], Tatjana Jakovska[2]

[1]Department of Clinical Microbiology, Institute for Respiratory Diseases in Children, Skopje, Republic of Macedonia; [2]Pediatric Department, Institute for Respiratory Diseases in Children, Skopje, Republic of Macedonia

Citation: Popova G, Boskovska K, Arnaudova-Danevska I, Smilevska-Spasova O, Jakovska T. Sputum Quality Assessment Regarding Sputum Culture for Diagnosing Lower Respiratory Tract Infections in Children. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1926-1930. https://doi.org/10.3889/oamjms.2019.551

Keywords: Sputum culture; Quality assessment; *Streptococcus pneumoniae; Escherichia coli*

***Correspondence:** Gorica Popova. Department of Clinical Microbiology, Institute for Respiratory Diseases in Children, Skopje, Republic of Macedonia. E-mail: gorica.popova@yahoo.com

Received: 03-May-2019; **Revised:** 17-Jun-2019; **Accepted:** 18-Jun-2019; **Online first:** 30-Jun-2019

Copyright: © 2019 Gorica Popova, Katerina Boskovska, Ivana Arnaudova-Danevska, Olga Smilevska-Spasova, Tatjana Jakovska. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The clinical relevance of specimens from the lower airways is often debatable. However, they are most commonly examined for diagnosing lower respiratory tract infections (LRTIs).

AIM: This study aimed to determine the diagnostic value of sputum quality assessment about sputum culture for diagnosing LRTIs in children.

METHODS: In six months, a total of 1485 sputum samples were quality assessed by using Bartlett's grading system. All samples, regardless of their quality, were cultured, identified, and antimicrobial susceptibility testing was performed by Kirby-Bauer disc-diffusion method.

RESULTS: Among the acceptable category, defined by Bartlett's grading system, 132 (63.2%) samples showed culture positivity of which *Streptococcus pneumoniae* 48 (36.4%) was most commonly isolated, followed by *Moraxella catarrhalis* 22 (16.7%) and *Haemophilus influenza* 21 (15.9%). Among the non-acceptable category, 185 (14.5%) samples were culture positive of which most commonly isolated were *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with 64 (34.6%), 54 (29.2%) and 28 (15.1%), respectively.

CONCLUSION: Sputum quality assessment is a useful tool for distinguishing the true respiratory pathogens from possible colonising flora for which antibiotic treatment should not be highly considered.

Introduction

Bacteriological examination of the specimens obtained from the lower respiratory tract is quite challenging for medical microbiologists. All expectorated sputa are contaminated by the oropharyngeal flora, which may include potential pathogens. The decision of whether the isolated pathogenic bacteria represent true causes of lower respiratory tract infection or are only colonisers of the upper respiratory tract is often impossible. This dilemma is particularly present when young children are in question because obtaining sputum with good quality from this age group is an especially difficult task. This is due to several reasons. First of all, instead of expectorating the sputum, children tend to swallow it and usually have difficulty producing an adequate amount of specimen [1]. Moreover, compared to adults, children have higher colonisation rate of the respiratory tract with bacteria such as *Streptococcus pneumoniae, Haemophilus influenza* and especially in infants, high colonisation rate with *Escherichia coli* [2], [3], [4]. The latter increases the likelihood of contamination of the sputum specimen during its collection.

Involving the method for assessment of the sputum quality allows us to estimate the amount of oropharyngeal contamination. This method is performed by microscopic examination of the cellular components in a stained smear of the specimen, seen under the low power field magnification (LPF). A presence of two cell-types: *s*quamous *e*pithelial *c*ells (SEC) and inflammatory cells, primarily polymorphonuclear leukocytes, is taken into consideration. SEC is found only in the upper respiratory tract, so this finding suggests oropharyngeal-contamination, whereas the presence of polymorphonuclear leukocytes, suggests material derived from the site of active infection [5].

There are several published criteria for assessing the quality of sputum. According to Murray and Washington [6] and Geckler et al., [7], sputum quality assessment should be dependent only on the presence of the SEC, seen microscopically at low-power field (LPF) magnification, regardless of the number of the white blood cells (WBC). On the other hand, Van Scoy [8] states that specimens with more than 25 leukocytes per LPF should be accepted regardless of the number of the SEC.

The variations in the thickness of the material in different areas of the same slid causes inconsistencies when only one type of cells is taken into account. This can be overcome by assessment of the sputum quality according to the WBC-SEC ratio, as Bartlett [9] recommends.

Heineman and Radano [10] describe a similar scheme for screening sputum specimens, and the criterion for acceptability is the presence of more than ten WBC per SEC, seen microscopically at LPF.

In the present study, 1485 sputum specimens were quality assessed according to criteria of Bartlett et al., [9] and these findings were compared with the results of the bacteriological examination of the same specimens.

Methods

The studied population was children suspected having lower respiratory tract infections, aged from 0 to 14 years, treated in ambulatory and hospital settings at the Institute for Respiratory Diseases in Children, Skopje.

In six months, from 01.07.2017 to 30.12.2017, a total of 1485 sputa were quality assessed after slides of the specimens had been gram-stained and microscopically examined at 100x magnification. The sputa quality was assessed according to the criteria of Bartlett et al., [9], based on the relative number of SEC and inflammatory cells, seen microscopically per LPF. According to these criteria, for every specimen, a Q-score (sum of "+" and "–"assigned values) was calculated, following the scheme: + 2 if > 25 WBC were seen per LPF, + 1 if 10-25 WBC were seen per LPF; on the other hand, - 2 was assigned if > 25 SEC were seen per LPF and - 1 if 10-25 SEC were seen per LPF. The "+" Q-score indicated material derived from the site of an active infection and these samples were categorized as acceptable. A "0" or "-" Q-score suggested low sputum quality, excessive oropharyngeal contamination and these samples were categorized as non-acceptable.

All sputum specimens, regardless of their quality, were further processed, using standard microbiological procedures for bacterial isolation and identification. Sputa were inoculated on the same day on 5% sheep blood agar, chocolate agar and *Candida albicans*-chromogenic agar. Plates were incubated at 37°C overnight in 5% CO_2 and were observed up to 48 hours. If no growth of relevant bacteria was seen, the cultures were declared negative. Preliminary identification of the bacteria was based on colony morphology and cultural characteristics on selective and differential media. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc-diffusion method according to the methodology proposed by the European Committee on Antimicrobial Susceptibility Testing [11]. Interpretation of zones of inhibition around the antibiotic discs was made according to EUCAST- breakpoint tables for interpretation of MICs and zone diameters [12].

The results of the bacteriological examination were evaluated against the assessment of the sputa quality - assigned as a value of the Q-score.

Results

The results of sputa quality assessment are given in Figure 1.

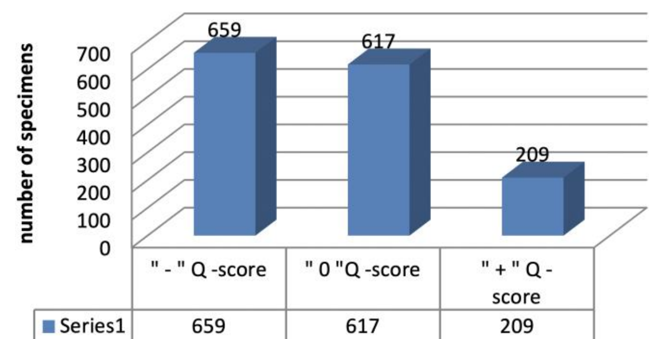

Figure 1: Sputa quality assessment

Based on Bartlett's screening criteria, out of 1485 processes sputum samples, 209 (14.1%) were good quality (acceptable category), and 1276 (85.9%) were low-quality (non-acceptable category). Of a total

of 209 sputa with good quality (acceptable category), 132 were culture positive (63.2%) and of 1276 sputa with low quality (non-acceptable category), 185 were culture positive (14.5 %), p = 0,000.

Distribution of isolated potential pathogenic bacteria from sputa with good quality (acceptable category) is given in Figure 2.

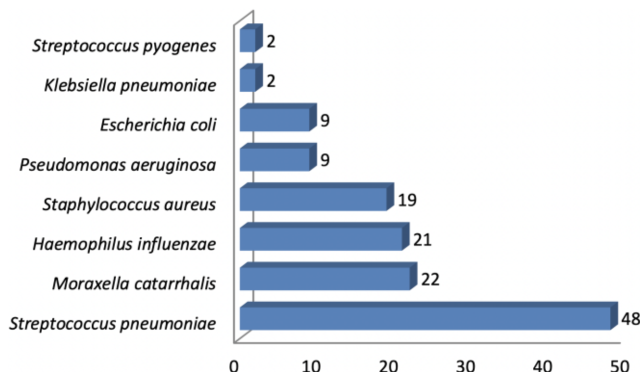

Figure 2: Distribution of isolated bacterial species from sputa with good quality (acceptable category)

The most frequently isolated bacteria from sputa with good quality (acceptable category) were *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* with 36.4%, 16.7% and 15.9%, respectively.

Distribution of isolated potential pathogenic bacteria from sputa with low quality (non-acceptable category) is given in Figure 3.

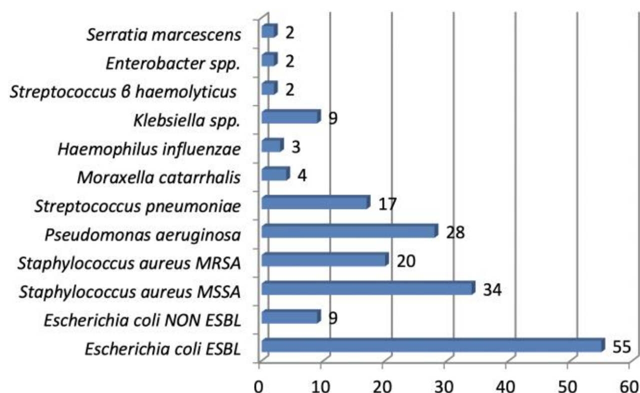

Figure 3: Distribution of isolated bacterial species from sputa with low quality (non-acceptable category); MSSA – Methicillin-Sensitive Staphylococcus aureus; MRSA – Methicillin-Resistant Staphylococcus aureus; ESBL – Extended Spectrum β-Lactamases

The most commonly isolated bacterium from low-quality sputum was *Escherichia coli* with 34.6% (64/185), of which ESBL (Extended Spectrum β-Lactamases)-producers were 85.9% (55/64). The second most often isolated was *Staphylococcus aureus* with 29.2% (54/185), of which MRSA (Methicillin-Resistant *Staphylococcus aureus*) was 37% (20/54).

Of all reported antimicrobial susceptibility test results, 23.6% (75/317) were reported for multidrug-resistant bacteria such as MRSA and ESBL-*Escherichia coli*, while microscopic examination of the specimens reviled low sputum quality.

Table 1 contains the number of isolated *Escherichia coli* and bacteria other than *Escherichia coli* from sputa with “0/-” Q-score (non-acceptable category) and sputa with “+” Q-score (acceptable category).

Table 1: The probability of *Escherichia coli* isolation in the function of sputum quality

	E. coli	Isolated Organisms other than E. coli
Non-acceptable category	64	121
Acceptable category	9	123

$$RR(relative\ risk) = \frac{64 : (64 + 121)}{9 : (9 + 123)} = 5.07$$

The low-quality sputum (as a risk factor) increases the relative risk for *Escherichia coli* isolation by 5 times.

Table 2 displays the number of isolated *Staphylococcus aureus* and bacteria other than *Staphylococcus aureus* from sputa with “0/-” Q-score (non-acceptable category) and sputa with “+” Q- score (acceptable category).

Table 2: The probability of *Staphylococcus aureus* isolation in the function of sputum quality

	S. aureus	Organisms other than S. aureus
Non-acceptable category	54	131
Acceptable category	19	113

$$RR(relative\ risk) = \frac{54 : (54 + 131)}{19 : (19 + 113)} = 2.07$$

Low sputum quality, as a risk factor, increases the relative risk for *Staphylococcus aureus* isolation by 2 times.

Discussion

The role of medical microbiologists is to isolate a causative agent of a certain infection which is highly dependent on the quality of the specimen. Among adults with pneumonia, about 60% can produce an adequate sputum specimen for microbiologic evaluation [13]. When infants and young children are considered having LRTIs, collecting good sputum specimen is hardly feasible. In this study, according to Bartlett’s grading system, the majority (nearly 86%) of the sputum specimens obtained from young children was low-quality. Therefore rejection of such a huge number of specimens tends to be complex. Most of them were declared negative (with

the remark that the specimens were with low quality, so the possibility for false negative results is not excluded). However, the biggest concern was 14.5% culture positive specimens (among non-acceptable category) where the majority of isolated bacteria were multidrug resistant (MDR) bacteria, such as ESBL-producing *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA). According to the results of this study, the probability for *Escherichia coli* isolation is 5 times increased if the sputum is low-quality, whereas the probability for isolating *Staphylococcus aureus* is doubled.

The isolation of MDR-organisms from low-quality sputum promotes unnecessary prescription of antibiotics, which should be overcome by combined efforts of clinicians and microbiologists.

It is noteworthy that 9% (17/185) of positive cultures in the non-acceptable category were identified as *Streptococcus pneumoniae*. The latter is due to the significant *Streptococcus pneumoniae* colonisation rate of the respiratory tract in childhood. According to Abdullahi et al., [3] nasopharyngeal pneumococcal carriage prevalence among groups aged from 0-4 years was 57% in contrast to 6.4% in groups aged from 10-80 years.

However, the greatest benefit of the sputum assessment was its assistance in the further interpretation of the sputum culture. Antimicrobial Susceptibility Test (AST) results were released for all potential pathogens isolated from low-quality sputa, but it was annotated that, according to the microscopic examination of the specimen, the isolated bacteria are probably part of the colonising flora of the upper respiratory tract. Hence, antibiotic treatment is very uncertain. From a clinical perspective, knowing whether the isolated bacteria are the cause of LRTI or they are merely colonising flora, could be salient in deciding whether the antibiotic therapy should be prescribed. Under these conditions, modified reporting, with the additional explanation that the isolated bacteria are probably part of colonising flora, could be a good practice. Moreover, automatic reporting of AST results could be excluded, similarly, as it is explained for asymptomatic bacteriuria in a work of Leis et al., [14]. This type of reporting will certainly lead to a more rational prescription of antimicrobial therapy.

The problem with the low quality of sputum is evident, and, perhaps, it may be overcome by introducing the procedures for the collection of induced sputum specimen [15]. According to results from Pneumonia Etiology Research for Child Health (PERCH) study, good quality sputum specimen can be collected from children with pneumonia, aged from 1 to 59 months, through saline nebulization induction [1], [15]. It has already been said that < 10 SEC per LPM is the best measure of induced sputum quality in children with pneumonia [15].

In contrast to sputa with low quality, where the culture positivity was 14.5%, sputa with good quality were culture positive in 63.2%, p = 0.00. Other studies are reporting similar culture positivity. In the studies of Anevlavis et al., [13], Rana et al., [16], Mariraj et al., [17], acceptable sputa were culture positive in 72%, 77% and 63%, respectively. According to the results of this study, the most frequently isolated was *Streptococcus pneumoniae* – 36.1%. This finding is in correlation with the results from other studies [15], [18], [19], [20], which implies that *Streptococcus pneumoniae* represents the most important respiratory pathogen in community-acquired lower respiratory tract infections in children.

References

1. Grant LR, Hammitt LL, Murdoch DR, O, Brien KL, Scott A. Procedures for Collection of Induced Sputum Specimens From Children. Clin Infect Dis. 2012; 54(S2):S140-5. https://doi.org/10.1093/cid/cir1069 PMid:22403228 PMCid:PMC3297553

2. Millar EV, Watt JP, Bronsdon MA, Dallas J, Reid R, Santosham M, O'Brien KL. Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal colonization among unvaccinated household members. Clin Infect Dis. 2008; 47(8):989-96. https://doi.org/10.1086/591966 PMid:18781875

3. Abdullahi O, Nyiro J, Lewa P, Slack M, Scott JA. The descriptive epidemiology of Streptococcus pneumoniae and Haemophilus influenzae nasopharyngeal carriage in children and adults in Kilifi district, Kenya. Pediatr Infect Dis J. 2008; 27(1):59-64. https://doi.org/10.1097/INF.0b013e31814da70c PMid:18162940 PMCid:PMC2382474

4. Popova G, Jankuloski D, Félix B, Boskovska K, Stojanovska-Dimzovska B, Tasic V, Blagoevska K. Pulsed-Field-Gel-Elecrophoresis used for typing of extended-spectrum-β-lactamases-producing Escherichia coli isolated from infant's respiratory and digestive system. Mac. Vet. Rev. 2018; 41:133-141. https://doi.org/10.2478/macvetrev-2018-0016

5. Wong LK, Barry AL, Horgan SM. Comparison of six different criteria for judging the acceptability of sputum specimens. J Clin Microbiol. 1982; 16:627-631.

6. Murray PR, Washington JA. Microscopic and bacteriologic analysis of expectorated sputum. Mayo Clin Proc. 1975; 50:339-344. https://doi.org/10.1080/00357529.1975.11762852

7. Geckler RW, Gremillion DH, McAllister CK, Ellenbogen E. Microscopic and bacteriological comparison of paired sputa and transtracheal aspirates. J Clin Microbiol. 1977; 6:518-527.

8. Van Scoy RE. Bacterial sputum cultures, a clinician's viewpoint. Mayo Clin Proc 1977; 52:39-41.

9. Bartlett JG, Breiman RF, Mandell LA, File TM. Community-acquired pneumonia in adults: guidelines for management. Clin Infect Dis. 1998; 26:811-838. https://doi.org/10.1086/513953 PMid:9564457

10. Heineman HS, Radano RR. Acceptability and cost savings of selective sputum microbiology in a community teaching hospital. J Clin Microbiol. 1979; 10:567-73.

11. Matuschek, E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect. 2014; 20:O255-O266. https://doi.org/10.1111/1469-0691.12373 PMid:24131428

12. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone

diameters. Version 6.1, 2016. http://www.eucast.org

13. Anevlavis S, Petroglou N, Tzavaras A, et al. A prospective study of the diagnostic utility of sputum Gram stain in pneumonia. J Infect. 2009; 59:83-9. https://doi.org/10.1016/j.jinf.2009.05.011 PMid:19564045

14. Leis JA, Rebic GW, Daneman SM, Lo P, Larocquen M, Shojania KG, McGeer A. Reducing antimicrobial therapy for asymptomatic bacteriuria among noncatheterized inpatients: a proof-of-concept study. Clin Infect Dis. 2014; 58(7):980-3. https://doi.org/10.1093/cid/ciu010 PMid:24577290

15. Murdoch DR, Morpeth SC, Hammitt LL, et al. Microscopic Analysis and Quality Assessment of Induced Sputum from Children with Pneumonia in the PERCH Study. Clin Infect Dis. 2017; 64(S3):S271-S279.

16. Rana A, Sharma A, Pandey G. Diagnostic value of sputum Gram's stain and sputum culture in lower respiratory tract infections in a tertiary care hospital. Int J Curr Microbiol App Sci. 2017; 6:4310. https://doi.org/10.20546/ijcmas.2017.607.448

17. Mariraj J, Surekha Asangi Y, Krishna S, Suresh Sonth B, Ramesh, Shanmugam. Sputum Gram,s stain assessment in relation to sputum culture for respiratory tract infections in a tertiary care hospital. J Clin Diag Res. 2011; 5(8):1699-1700.

18. Khan S, Priti S, Ankit S. Bacteria Etiological Agents Causing Lower Respiratory Tract Infections and Their Resistance Patterns. Iran Biomed J 2015; 19(4):240-6.

19. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ. 2008; 86(5):408-16. https://doi.org/10.2471/BLT.07.048769 PMid:18545744 PMCid:PMC2647437

20. Rudan I, O'Brien KL, Nair H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health. 2013; 3(1):010401. https://doi.org/10.7189/jogh.03.010101