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Original Article

Utility of multiplex real-time polymerase chain reaction for the detection of bacteria from sputum samples of community acquired pneumonia patients in Dhaka, Bangladesh

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ABSTRACT

Background: This study was carried out to evaluate the utility of multiplex real-time polymerase chain reaction (PCR) to identify the common bacterial agents of community acquired pneumonia (CAP).

Methods: Sputum and blood samples were collected from 80 clinically suspected CAP patients in three tertiary-level hospitals in Dhaka city. Multiplex real-time PCR assay was carried out to simultaneously detect five common bacterial agents of CAP; *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila.* Routine microbiological methods and serology were carried out. The results of PCR were compared with culture, Gram stain and serology.

Results: Among the 80 patients, sputum samples of 35 (43.7%) patients were positive by PCR, of which the most commonly detected bacteria were *S. pneumoniae* (25/35, 71.4%), followed by *H. influenzae* (9/35, 25.7%) and *L. pneumophila* (1/35, 2.9%). All 80 sputum samples were negative for both *M. pneumoniae* and *C. pneumoniae* by PCR. Out of the 26 culture positive sputum samples, 8 (30.7%) were positive for *S. pneumoniae* and 1 (3.8%) was positive for *H. influenzae*. Among the 52 Gram stain valid sputum samples, 24 (46.1%) were *S. pneumoniae* and 7 (13.5%) were *H. influenzae*. By serology, out of the 80 cases, *M. pneumoniae* was detected in 32 (40%) and *C. pneumoniae* in 24 (30%) of cases. Mixed infections comprised of 38.8% (31/80) cases.

Conclusion: Multiplex real-time PCR is useful for the rapid and simultaneous detection of bacterial pathogens of CAP in sputum and can help support traditional laboratory methods for the accurate diagnosis of CAP patients.

Keywords: multiplex real-time PCR, community acquired pneumonia, *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila*

INTRODUCTION

Community acquired pneumonia (CAP) is a clinical syndrome where acute infection of the lungs develops in individuals not recently hospitalized and having no regular exposure to the health care system. CAP continues to be a major threat, especially among children, the elderly and compromised hosts such as people with underlying chronic diseases.[1] Globally, the incidence of CAP is estimated between 1.5 - 14 cases per 1000 person-years, and is affected by population characteristics, season and geography.[2] Studies have identified *Streptococcus pneumoniae* as the primary cause of CAP.[3] Lim et al. in their study found

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HIGHLIGHTS

1. **This is the first study in Bangladesh evaluating the utility of multiplex real-time PCR assay for the detection of common bacterial agents of community acquired pneumonia (CAP).**
2. **Multiplex real-time PCR assay supplemented with the traditional laboratory methods resulted in a higher microbial detection rate and detection of cases with mixed infections.**
3. **Multiplex real-time PCR assay can support the traditional laboratory methods for the accurate and early diagnosis of CAP patients.**

that the most common agent producing CAP are *Streptococcus pneumoniae* (48% of cases), followed by *Haemophilus influenzae* (20%), viruses (19%), *Chlamydophila pneumoniae* (13%), and *Mycoplasma pneumoniae* (3%).[4]

The cornerstone of CAP management is antimicrobial therapy where the therapeutic choices are influenced by the probable aetiologies, local pathogen resistance patterns, as well as patient factors.[5] Therefore, there is a good rationale for establishing the identity of pathogens causing disease in order to select antimicrobial agents against a specific pathogen, limit the misuse of antibiotics and its consequences, and identify infectious agents associated with notifiable diseases such as Legionnaires' disease or tuberculosis.[6]

In the etiological diagnosis of CAP, routine sputum Gram stain and conventional culture (gold standard method) have limitations and can yield inconclusive results in cases where patients are unable to produce an adequate specimen or had received antibiotics prior to assessment.[1,7] Cell cultures for the detection of *C. pneumoniae* and *M. pneumoniae* are labor-intensive, expensive, require specialist labs, grow slowly and lack sensitivity. Serology usually requires documentation of a rising antibody titre from acute phase to convalescent phase.[8] Therefore, with conventional techniques, a retrospective diagnosis is frequently the only option due to these challenges and associated delayed results.[9]

Molecular methods on the other hand, such as polymerase chain reaction (PCR) assay, offer a better approach for the rapid diagnosis of CAP.[10] PCR for gene amplification has made it possible to detect low number of infectious agents or even fragments of Deoxyribonucleic acid (DNA) from these agents with high sensitivity and specificity.[1] Multiplex real-time PCR technology allows the monitoring of in vitro DNA amplification successively, eliminating nonspecific amplification and the need for gel electrophoresis and provides results within 4-5 hours which decreases the risk of false positive results.[11] Furthermore, this technique offers the ability to detect multiple pathogens simultaneously, which is particularly economical for small volume samples.[12]

To the best of our knowledge, no study has been conducted from Bangladesh on the utility of multiplex real-time PCR for the detection of the causative agents of CAP. Therefore, the present study were carried out to evaluate the utility of multiplex real-time PCR assay to identify the common bacterial agents of CAP namely *S. pneumoniae*, *H. influenzae*, *C. pneumoniae*, *M. pneumoniae* and *Legionella pneumophila* from the sputum sample of patients suffering from CAP attending three tertiary care hospitals in Dhaka, Bangladesh. Additionally, the results of multiplex real-time PCR were compared with the findings of culture, Gram stain and serology.

METHODS

A. Study Population

Following purposive sampling techniques, sputum and blood samples were collected from 80 clinically diagnosed pneumonia patients from the respiratory medicine outdoor and general medicine indoor of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh; respiratory medicine outdoor of Dhaka Medical College Hospital (DMCH), and from National Institute Diseases of the Chest and Hospital (NIDCH). The study was carried out from March 2016 to February 2017 and all laboratory work was performed in the Department of Microbiology and Immunology of BSMMU. The inclusion and exclusion criteria previously described by Lim et al.[4] was used in this study to select the study population. Adult patients (age >18 years) with a provisional diagnosis of CAP fulfilling the inclusion criteria for pneumonia were included. Prior verbal and written consents were taken from the patients.

B. Sample collection and analysis

Sputum and blood samples were collected from each patient following standard laboratory procedure. Blood samples were processed to extract serum and stored at -20°C temperature for 4 - 5 months for serological assay.

C. Microbiological methods

Identification of bacteria was done by culture (colony morphology), Gram stain, biochemical tests and satellitism (when necessary). A presumptive etiology was considered if growth of a predominant bacterial pathogen from culture of sputum in combination with similar findings on Gram staining was present.[13-17]

C1. Culture

Sputum samples were inoculated on blood agar media, chocolate agar media and Maconkey's agar media (Oxoid Limited, Basingstoke, England) following standard microbiological protocols and incubated at 37°C temperature. Colony morphology of the bacterial growths was examined.[13,14]

C2. Gram staining of sputum

All sputum specimens were Gram stained and examined microscopically for the presence of white blood cells (WBCs), epithelial cells and bacteria. The slides were initially evaluated for quality under low-power field (10×) microscopic view. Purulence was measured by microscopy and was acceptable if there was >25 WBCs and <10 squamous epithelial cells per low-power field microscopic view. Then the bacterial morphological types were screened at oil immersion field (100×). The presence of a single morphotype of bacteria more than 10 in number per oil immersion field was defined as predominant bacteria. Results from sputum cultures were only considered significant if the above Gram-stain criteria were satisfied.[15-17]

D. PCR

Bacterial DNA extraction from sputum samples was carried out using Ribo-spinv RD (RNA/DNA) nucleic acid extraction kit (Geneall, Seoul, South Korea) according to the manufacturer's instructions. After extraction, the purified DNA was stored at -20°C temperature in micro centrifuge tubes for later analysis by multiplex real-time PCR assay. AllplexTM Respiratory Panel 4 (Seegene Inc. Seoul, South Korea) was used for multiplex real time PCR assay according to the manufacturer's instructions for simultaneous detection of five bacterial pathogens; *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila* from sputum sample.

The target genes used in this study for detection of the five bacterial pathogens were previously described by Park et al.[18] Multiplex real-time PCR assay was carried out using CFX96TM real-time PCR system (Bio-Rad Laboratories Inc., Hercules, California, United States) according to the manufacturer's instructions. The findings of CFX96TM real-time PCR system (Bio-Rad Laboratories Inc., Hercules, California, United States) displayed two graphs graph 1 **(FIGURE 1A)** and graph 2 **(FIGURE 1B)** showing the results of the multiplex real -time PCR assay. Interpretation of amplification curves of *S. pneumoniae*, *H. influenzae*, *M. pneumoniae* and internal control (IC) was done in graph 1, and that of *C. pneumoniae* and *L. pneumophila* was done in graph 2.

The results were interpreted as follows: samples with cycle threshold (Ct) value ≤42 were interpreted as target gene detected and those with Ct value >42 were interpreted as target gene not detected.

E. Serology

Indirect immune-enzyme assay (ELISA: enzyme-linked immunosorbent assay) was carried out for the 80 serum samples. Semi-quantification of immunoglobulin G IgG antibodies against *C. pneumoniae* using Chlamydophila Pneumonia ELISA IgG kit (Vircell, Microbiologist, Granada, Spain) and the detection of IgG antibodies against *M. pneumoniae* using Mycoplasma Pneumonia ELISA IgG kit (Vircell, Microbiologist, Granada, Spain) was carried out according to the manufacturer's instructions.

F. Data analysis and Statistics

Patient information was obtained through questionnaire and from the clinical record files of the patients. All data were entered into a database using Statistical Package for the Social Sciences Version-21. The results of multiplex real-time PCR were compared with culture, Gram stain and serology. The efficacy of multiplex real-time PCR in comparison to culture (gold standard method) and Gram stain was determined by

TABLE 1 Results of different laboratory testing methods for detection of bacteria among the study population (n=80)

Name of test	Clinical sample tested	Number of cases positive for bacteria n (%)	Number of cases negative for bacteria n (%)
Multiplex real-time PCR	sputum	35 (43.7)	45 (56.3)
Culture	sputum	26 (32.5)	54 (67.5)
*Gram stain	sputum	52 (65.0)	28 (35.0)
ELISA for IgG	serum	32 (40.0)	48 (60.0)

*Gram stain result was interpreted as positive when: pus cell >25/LPF with or without predominant bacteria

calculating the diagnostic parameters: sensitivity, specificity, positive predictive value, and negative predictive value by using standard equations.

RESULTS

Out of the 80 CAP cases in sputum sample; 35 (43.7%) were positive for bacteria by multiplex real-time PCR, 26 (32.5%) by conventional culture and 52 (65.0%) by Gram stain and in blood sample; by serology (ELISA for IgG), 32 (40.0%) cases were positive **(TABLE 1)**.

TABLE 2 shows the distribution of different bacterial agents among positive cases by different tests. All the cases were negative by PCR for both *M. pneumoniae* and *C. pneumoniae*. In serology, among the 32 ELISA (IgG) positive cases, eight cases were positive for *M. pneumoniae* IgG antibodies only and 24 cases were positive for both *M. pneumoniae* and *C. pneumoniae* IgG antibodies. **TABLE 3** shows the frequency of detection of the different types of bacteria by multiplex real-time PCR, culture and serology (ELISA for IgG).

Single and mixed (polymicrobial) infection detected by multiplex real-time PCR, culture and serology among study population is shown in **TABLE 4**. Out of the 80 CAP cases, 31 (38.8%) single bacterial, 16 (20.0%) double bacterial and 15 (18.8%) triple bacterial cases were detected. Mixed (polymicrobial) infection comprised of 31 (38.8%) cases which was the sum of double and triple bacterial infection cases.

The correlation of the results of *S. pneumoniae* and *H. influenzae* by multiplex real-time PCR in comparison to results of culture and Gram stain is shown in **TABLE 5**.

TABLE 2 Distribution of the different bacteria detected by different laboratory tests

Name of bacteria detected	Number of bacteria detected n (%)
Multiplex real-time PCR (n=35)	
S. pneumoniae	25 (71.4)
H. influenzae	09 (25.7)
L. pneumophila	01 (2.9)
Culture (n=26)	
S. pneumoniae	08 (30.7)
H. influenzae	1 (3.8)
S. aureus	05 (19.2)
K. pneumoniae	09 (34.6)
E. coli	01 (3.8)
Pseudomonas species	02 (7.6)
Gram stain (n=52)	
Predominantly Gram positive diplococci, suggestive of *S. pneumoniae*	24 (46.1)
Predominantly Gram negative coccobacilli, suggestive of *H. influenzae*	07 (13.5)
Predominantly Gram positive cocci	05 (9.6)
Predominantly Gram negative bacilli	10 (19.2)
Others (upper respiratory flora)	06 (11.3)
ELISA (IgG) (n=32)	
M. pneumoniae	32 (100.0)
C. pneumoniae	24 (75.0)

Values are expressed as absolute number and percentage over row total

In this study, the efficacy of multiplex real-time PCR in comparison to culture and Gram stain was seen. Considering culture as gold standard, the sensitivity of multiplex real-time PCR was 100% and specificity was

TABLE 3 Frequency of detection of the different types of bacteria by multiplex real-time PCR, culture and serology (ELISA for IgG)

Name of bacteria	Only PCR positive n (%)	Only Culture positive n (%)	Both PCR and culture positive n (%)	Only ELISA (IgG) positive n (%)
S. pneumoniae (n= 25)	17 (68.0)	0	8 (32.0)	-
H. influenzae (n= 9)	8 (88.9)	0	1 (11.1)	-
M. pneumoniae (n=32)	0	-	-	32 (100.0)
C. pneumoniae (n=24)	0	-	-	24 (100.0)
L. pneumophila (n= 1)	1 (100.0)	-	-	-
K. pneumoniae (n= 9)	-	9 (100.0)	-	-
S. aureus (n=5)	-	5 (100.0)	-	-
Pseudomonas species (n=2)	-	2 (100.0)	-	-
E. coli (n=1)	-	1 (100.0)	-	-

(-), not done
Values are expressed as absolute number and percentage over row total

TABLE 4 Single and mixed (polymicrobial) infection detected by multiplex real-time PCR, culture and serology among the study population (n=80)

Type of bacterial infection detected Name of bacteria	Number of cases n (%)
Single bacteria	
S. pneumoniae	12 (15.0)
H. influenzae	1 (1.3)
M. pneumoniae	4 (5.0)
K. pneumoniae	8 (10.0)
S. aureus	3 (3.7)
E. coli	1 (1.3)
Pseudomonas species	2 (2.5)
Total	31 (38.8)
Two bacteria	
S. pneumoniae plus *H. influenzae*	2 (2.5)
S. pneumoniae plus *M. pneumoniae*	2 (2.5)
H. influenzae plus *M. pneumoniae*	2 (2.5)
M. pneumoniae plus *C. pneumoniae*	9 (11.2)
S. pneumoniae plus *K. pneumoniae*	1 (1.3)
Total	16 (20.0)
Three bacteria	
S. pneumoniae, *M. pneumoniae* plus *C. pneumoniae*	8 (10.0)
H. influenzae, *M. pneumoniae* plus *C. pneumoniae*	4 (5.0)
L. pneumophila, *M. pneumoniae* plus *C. pneumoniae*	1 (1.3)
S. aureus, *M. pneumoniae* plus *C. pneumoniae*	2 (2.5)
Total	15 (18.8)

64.8%, positive predictive value was 26.5%, negative predictive value was 100% and accuracy was 68.8%. In relation to Gram stain sensitivity, multiplex real-time PCR was 96.8%, specificity 91.8%, positive predictive value 88.2%, negative predictive value 97.8% and accuracy 93.8%.

TABLE 5 Correlation of results of *S. pneumoniae* and *H. influenzae* by multiplex real-time PCR in comparison to results of culture and Gram stain

Multiplex real-time PCR	Culture		Gram stain	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Positive (n= 34)	9 (26.5)	25 (73.5)	30 (88.2)	4 (11.8)
Negative (n=46)	0	46 (100.0)	1 (2.2)	45 (97.8)
Total 80	9 (11.3)	71 (88.8)	31 (38.8)	49 (61.3)

Values are expressed as absolute number and percentage over row total; PCR - Polymerase chain reaction

DISCUSSION

This study was undertaken to report on the utility of multiplex real-time PCR assay for the identification of common bacterial agents of CAP. A bacterial etiology of CAP was established for 35 (43.7%) out of the 80 CAP patients by multiplex real-time PCR assay from sputum samples. The findings are consistent with Hohenthal et al. which reported 54.4% detection rate by PCR.[19] However, Johansson et al. found 67.0% (124 out of 184) sputum samples to be positive by PCR for *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis* which was higher than our findings.[20] In this study, the most frequently detected bacteria by multiplex real-time PCR was *S. pneumoniae* (71.4%). *S. pneumoniae* continues to be the most frequently identified pathogen associated with CAP.[21] The results are in concordance with studies from different countries which found *S. pneumoniae* as the predominant pathogen in respiratory samples by PCR. However, the detection rate of *S. pneumoniae* in those studies ranged from 21.7% to 38.0% which is lower than our data.[16,19,20,22] *H. influenzae* (25.7%) was the second most frequently detected bacteria by PCR in our study. Aydemir et al. reported *H. influenzae* in 31.0% of samples by PCR that is similar to our results.[22]

L. pneumophila was positive in the specimen of one patient (2.9%) only by PCR. Aydemir et al. from Turkey reported that *L. pneumophila* was not detected in their study which coincides with our findings.[22] Moreover, other researchers have found that the frequency of CAP due to *L. pneumophila* was relatively low in Asian communities.[17] On the other hand, the frequency of Legionella infection was 6.0% in some studies, while in another study, a higher rate of 15.0% have been reported.[4, 23, 24]

All sputum specimens were negative (0%) for both *M. pneumoniae* and *C. pneumoniae* by multiplex real-time PCR assay. Herrera et al. explained the very low positivity of PCR results for *M. pneumoniae* and *C. pneumoniae* might be due to several reasons; the bacterial load being below the detection limit for the PCR assay, previous antibiotic treatment in patients, dilutions of samples, degradation of significant amounts of DNA during the sample storage process, the presence of interfering DNA coming from human cells or other colonizing microorganisms of the

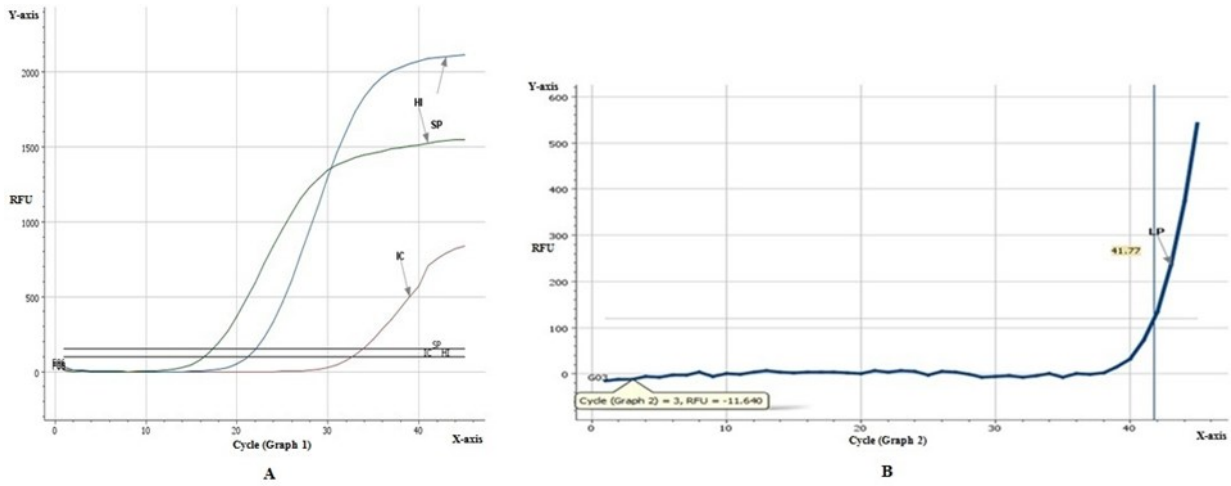

FIGURE 1 Results of multiplex real-time PCR of sputum sample showing in Graph 1 (A) amplification of *S. pneumoniae* (SP, blue curve), *H. influenzae* (HI, green curve) and internal control (IC, red curve) and in Graph 2 (B) amplification of *L. pneumophila* (LP, blue curve). The X and Y axes represents amplification cycles and relative fluorescence units (RFU), respectively

respiratory tract, and primer mismatches due to strain variations at the primer recognition site, which can affect amplification. Our negative results by PCR for *M. pneumoniae* and *C. pneumoniae* coincides with the study of Herrera et al.[25] There is another explanation of PCR negativity which is that PCR results are more useful during the early stages of infection when more organisms are likely to be present. However, the likelihood of positivity in PCR result diminishes overtime because the sensitivity decreases significantly in the interval from symptom onset to specimen collection increases.[26] There is another opinion that PCR detection of atypical bacteria has limitations since no consensus exists regarding which molecular target should be amplified to achieve higher sensitivity and specificity neither does a clearly defined standard protocol exist.[27]

A total of 26 (32.5%) patients were positive and 54 (67.5%) were negative in sputum culture. Shah et al. reported 29% isolation rate of bacteria from sputum of CAP patients in India which was comparable to this study.[28] In contrast, Gutirrez et al. reported 55.5% culture positivity in Spain which is higher than our findings.[29]

Out of the 26 culture positive patients, 8 (30.7%) were positive for *S. pneumoniae* and 1 (3.8%) was positive for *H. influenzae*. The isolation rate of *S. pneumoniae* among CAP patients was 23.0% in a study from Japan which was in concordance with our findings.[30] By contrast, *S. pneumoniae* was isolated at lower rates in Spain (16.8%), while Johnson et al. reported 42% isolation rate of *S. pneumoniae* in Australia which is higher than ours.[29,31] Studies have shown the isolation rate of *H. influenzae* was 7.4% in Japan and 9% in Australia which is similar to our results.[30, 31]

In our study, 52 (65.0%) samples had valid findings by Gram stain and in 46 (57.5%) samples, a predominant organism was identified. In the study by Ewig et al., valid sputum was obtained in 39.0% of patients and a predominant organism in only 31.0% cases by Gram stain and in the study by García-Vázque et al., a predominant morphotype was identified in only 45.0% of the patients; however, their results are lower compared to our data.[32, 33]

A total of 32 (40.0%) blood samples were positive by serology for *M. pneumoniae* and *C. pneumoniae*, however both organisms were not detected by PCR. Results of serologic tests could not be used to resolve the discrepancies in the PCR findings of our study, since only a single serum sample was available from the CAP patients. Furthermore, no serology assay kits were available for the detection of immunoglobulin M (IgM) antibodies against *M. pneumoniae* and *C. pneumoniae* during the study period. Thurman et al. found that among 77 patients with negative real-time PCR results, 54.0% was positive by serology which concur with our data.[26] *M. pneumoniae* was detected in 40.0% (32 out of 80) cases and *C. pneumoniae* was positive in 30.0% (24

out of 80) cases. Studies have found the sensitivity of *M. pneumoniae* detection by PCR in CAP patients to be generally low, being more useful during early stages of infection when a higher microbial count is likely to be present and proper sample collection, handling and processing is crucial for the diagnosis of *M. pneumoniae* pneumonia by PCR.[26, 34] The results of *C. pneumonia* by PCR in this study are also in agreement with earlier reports.[19, 34]

In our study, the combined results of multiplex real-time PCR assay, culture and serology showed that 31 (38.8%) cases were found to have CAP due to mixed (polymicrobial) infections. Similarly, more than one causative pathogen was detected in 35.0% of cases by Templeton et al. and 40.0% cases of mixed infections was reported by Johansson et al.[20,23] On the other hand, Saito et al. observed mixed infection in 25.9% cases, which is lower than our findings.[35] There was a wide variety of combinations of pathogens found, the most frequent in 18 cases (22.5%) being a typical bacterial pathogen plus an atypical organism, which is in agreement with the findings by Lim et al. where it was 21.0%.[4] *S. pneumoniae* was involved in 13 cases out of the 31 mixed infections (41.9%). Corresponding rates of detection of *S. pneumoniae* in different studies ranged between 54% to 64.3%.[29, 36] The high rates of mycoplasma and chlamydia co-infections found in our study are similar with the findings of Bao et al.[37]As both of these pathogens were serologically diagnosed instead of sputum culture, a false-positive result could not be excluded.

By using multiplex real-time PCR assay compared to culture method, the pathogen detection rate of *S. pneumoniae* increased from 30.7% to 71.4%, *H. influenzae* raised from 3.8% to 25.7%, and detection of dual pathogen presence increased from none to two patients. The study conducted by Aydemir et al. reported that by using PCR compared to culture, the pathogen detection rate increased from 13% to 35.0% in *S. pneumoniae*, from 20% to 46% in *H. influenzae* which coincide with our findings, however their detection of dual pathogen presence increased from 2 to 20 patients which was higher than our data.[22] Mustafa et al. reported that after antibiotic treatment, culture of *S. pneumoniae* become negative quickly but PCR remain positive and this might be a possible explanation for our findings.[16]

Considering culture as gold standard, the sensitivity of multiplex real-time PCR was 100.0% and specificity was 64.8%, positive predictive value was 26.5%, negative predictive value was 100% and accuracy was 68.8%. These findings coincide with the findings by Yang et al. who found 90.0% sensitivity but 80.0% specificity which is higher than the present study.[38] Similarly, Abdeldaim et al. found sensitivity in 95.0% cases and specificity in 75.0% cases.[39] Morozumi et al. reported multiplex PCR sensitivity in 96.0% and specificity in 93.2% cases.[1] All these studies showed similar sensitivity but higher specificity than the present study. In contrast, Stralin et al. found 92.0% sensitivity which was similar to our data but found lower specificity (42.0%) than the present study, however they did not give any explanation about the reason.[40] The 64.8% specificity that we found may be due to small sample size or might be due to growth of fastidious organisms in culture. Correlating Gram staining, the sensitivity of multiplex real-time PCR was 96.8% and specificity was 91.8%, the positive predictive value was 88.2%, negative predictive value was 97.8% and accuracy was 93.8%.

Conclusion

Our results show that by supplementing the traditional laboratory methods with multiplex real-time PCR assay for detection of the common bacterial agents of CAP, a higher microbial yield and detection of mixed infections was achieved. Therefore, this technology can be an important additional diagnostic tool for the accurate and early diagnosis of CAP patients in this region.

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Author Contributions

- Conception and design: SA, FMA, RUS, MRAM
- Acquisition, analysis, and interpretation of data: SA, FMA, RUS, MRAM
- Manuscript drafting and revising it critically: SA, FMA, RUS, MYA, RP, AK, KMTS, ANIS, MRAM
- Approval of the final version of the manuscript: SA, FMA, RUS, MRAM
- Guarantor accuracy and integrity of the work: SA

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Conflict of Interest

The authors declare no conflicts of interest.

Ethical approval

Ethical approval for this study was granted by the Institutional Review Board of BSMMU (memo number: BSMMU/2016/8047).

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