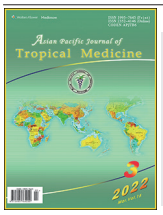

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Antimicrobial susceptibility and serotypes of *Neisseria meningitidis* and *Streptococcus pneumoniae* in Sri Lanka: Experience from the National Reference Laboratory

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ABSTRACT

Objective: To determine the antimicrobial susceptibility and serotypes of *Neisseria (N.) meningitidis* and *Streptococcus (S.) pneumoniae* in Sri Lankan patients.

Methods: We retrospectively analyzed 11 blood culture specimens from suspected patients with invasive meningococcal disease and 26 *S. pneumoniae* clinical isolates. We tested 6 antimicrobials against *N. meningitidis* and 12 antimicrobials against *S. pneumoniae*. Meningococcal serogroup was determined by realtime PCR and Quellung serotyping was used for pneumococcal analysis.

Results: *N. meningitidis* serogroup B was the most common in this study. Intermediate-susceptibility to penicillin was seen in 75.0% (6/8) of strains. Susceptibility to ciprofloxacin, levofloxacin and cotrimoxazole was 62.5% (5/8), 62.5% (5/8) and 87.5% (7/8), respectively. Excellent susceptibility was seen in cefotaxime and meropenem. In *S. pneumoniae*, the most common serotype was 19F in both invasive and non-invasive pneumococcal diseases. The majority of strains showed multidrug resistance. Penicillin non-susceptibility in non-meningeal strains were 13.6% and all meningeal strains were penicillin resistant. Erythromycin was highly resistant in both groups. Amoxicillin showed excellent susceptibility in non-invasive pneumococcal diseases strains. Linezolid, levofloxacin and vancomycin showed 100.0% susceptibility in all pneumococcal isolates.

Conclusions: Implementation of vaccines should be considered, especially for children and high-risk populations. This may contribute to reducing pneumococcal and meningococcal invasive disease burden and help prevent emergence of antimicrobial resistant strains.

KEYWORDS: *Neisseria meningitidis*; *Streptococcus pneumoniae;* Sri Lanka; Antimicrobial resistance; Serotypes; Invasive bacterial diseases

Significance

Serogroups and antimicrobial susceptibility of Sri Lankan meningococcal isolates were presented and two clusters linked epidemiologically were described in this paper. This information is important for choice of empiric therapy, vaccine selection and outbreak prevention. Data on pneumococcal characterization is presented, to support introduction of appropriate vaccine in the national program to prevent morbidity, mortality and emergence of resistant pneumococcal strains.

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1. Introduction

Neisseria (N.) meningitidis and *Streptococcus (S.) pneumoniae* are two important pathogens in focus at the World Health Organization (WHO)'s 'Defeating meningitis by 2030: global roadmap'. In Sri Lanka, pneumonia and meningitis are two important causes of infant and childhood mortality[1,2]. Early antimicrobial therapy has shown to reduce mortality and morbidity of these deadly diseases[3,4]. Meningitis by all-cause is a notifiable disease in Sri Lanka, though pathogen-specific surveillance is not in place.

Lack of country-specific data and high vaccine cost were implicated in the delay of vaccine implementation worldwide[5]. In Sri Lanka, knowledge on circulating serotypes and antimicrobial resistance in pneumococcal and meningococcal diseases is limited or not known.

N. meningitidis and *S. pneumoniae* cause acute bacterial meningitis, pneumonia and bacteremia from mild to life-threatening sepsis[3,4]. Though not highly contagious, close contact in both diseases can cause smaller outbreaks in crowded living conditions to epidemics[3,4]. High asymptomatic nasopharyngeal colonization is associated with crowding, close contact, smoking and respiratory infections[3,4].

The burden of pneumococcal disease remains high among children[6]. *S. pneumoniae* is the major cause of morbidity and mortality associated with childhood bacterial pneumonia worldwide[7]. Despite advances, 5%-10% mortality in early pneumococcal disease has remained constant[4]. WHO estimated 0.7 to 1 million children aged under 5 years die from pneumococcal disease every year[8].

Over 100 serotypes of *S. pneumoniae* differ in their invasiveness, patient age, geographical distribution, carriage and antimicrobial resistance[4,9]. Growing antimicrobial resistance has shown to increase pneumococcal disease burden[8]. In Sri Lanka, multidrug-resistant (MDR) strains causing fatal infant pneumococcal meningitis has been previously reported[10].

Approximately 1.2 million cases of invasive meningococcal disease occur yearly worldwide, with a case fatality rate ranging between 4.1%-20.0%[6]. The incidence of invasive meningococcal diseases in Asia-Pacific region ranges from 0.02 to 0.20 cases per 100000 persons per year. However, the number of cases has shown to be higher in certain countries and specific sub-populations and the burden is high among children and adolescents[6]. There are 12 known serogroups of *N. meningitidis*, but commonly six serogroups (A, B, C, W135, X, Y) are associated with most invasive meningococcal disease cases worldwide[4]. In Sri Lanka, sporadic invasive meningococcal disease cases have been previously reported[1].

Awareness of country-specific circulating serotypes is important in selecting suitable vaccines as they are serogroup or serotype-specific. Although pneumococcal serotyping and their susceptibility profile have been studied previously, this is the first publication on the characterization of *N. meningitidis* in Sri Lankan patients to our best knowledge.

Our aim was to determine the serotypes and antimicrobial susceptibility of *N. meningitidis* and *S. pneumoniae* in Sri Lankan strains received by the National Reference Laboratory.

2. Materials and methods

2.1. Study population

We retrospectively analyzed 26 *S. pneumoniae* clinical isolates, received between September 2019 and February 2020 and 11 blood culture specimens from suspected-invasive meningococcal disease patients received between December 2019 to February 2020. All these specimens were received at the National Reference Laboratory (NRL), Department of Bacteriology, Medical Research Institute, Sri Lanka either to confirm pathogen identification, antimicrobial susceptibility testing or as a routine clinical investigation. According to laboratory records, highest number of clinical specimens for suspected invasive meningococcal disease were received during this period.

All specimens were transported in room temperature; bacterial isolates were in sealed containers in agar medium and blood were in automated blood culture bottles. These specimens were sub cultured and confirmed at NRL. All specimens were stored and maintained at -70 ℃ in cryo vials. The sociodemographic, clinical and laboratory data were extracted from laboratory records.

2.2. Pathogen identification

These isolates were identified preliminarily by typical colony morphology, Gram stain and biochemical tests. *S. pneumoniae* was confirmed by bile solubility, optochin disk test and by automated BD Phoenix® system.

All 11 blood cultures obtained from suspected-invasive meningococcal disease patients yielded growth at the time of primary subculture and *N. meningitidis* was confirmed by realtime PCR. Out of the 11 clinical specimens of *N. meningitidis*, we were able to retrieve only 8 isolates.

2.3. Antimicrobial susceptibility testing (AST)

For *N. meningitidis* clinical isolates (n=8), penicillin and

levofloxacin minimum inhibitory concentrations (MIC) were determined by automated BD Phoenix® and *E*-test respectively; disk diffusion was performed for cefotaxime, meropenem, cotrimoxazole and ciprofloxacin.

For *S. pneumoniae* clinical isolates (*n*=26), penicillin, cefotaxime, meropenem, vancomycin, linezolid, amoxicillin, cefepime, erythromycin, tetracycline, cotrimoxazole, clindamycin and levofloxacin MIC were determined by automated BD Phoenix®.

Clinical and Laboratory Standard Institute (CLSI) M100, 31st edition was used for performance and interpretation of these pathogens[11]. Identification and antimicrobial susceptibility testing were performed at the National Reference Laboratory, Department of Bacteriology, Medical Research Institute, Sri Lanka.

2.4. Serotyping

Totally, 26 pneumococcal cultures of *S. pneumoniae* were serotyped by Neufeld's Quellung reaction method using type-specific antisera (Staten's Serum Institute, Denmark). Meningococcal serogroups were analyzed by realtime PCR with serogroup-specific primers for serogroups A, B, C, W135, X and Y in 11 *N. meningitidis* specimens. These were performed at the WHO South East Asia Reference Laboratory for Invasive Bacterial Diseases, Christian Medical College, Vellore, India.

2.5. Ethical approval

Ethics approval was obtained from the Ethics Review Committee, Medical Research Institute Colombo, Sri Lanka under project no. 08/2020.

3. Results

3.1. N. meningitidis

A total of 11 specimens (8 blood culture isolates, 3 venous blood) from suspected-invasive meningococcal disease patients were confirmed by realtime PCR.

The patients' age ranged from 43 days to 56 years (median: 33 years) with male predominance. They were from hospitals in the Western (*n*=7), North-Central (*n*=1), Eastern (*n*=2) and Northern (*n*=1) Provinces. There were 3 prison inmates in this cohort.

3.1.1. Clinical information

Meningococcemia was present in all 11 patients and 45.5% (*n*=5) developed shock. Petechial or purpuric rash was seen in 54.5% (*n*=6) and meningitis was diagnosed in 45.5% (*n*=5) of the patients.

Three patients with invasive meningococcal disease died within 48 hours of hospital admission with meningococcemia followed by shock. One patient also had meningitis. The case fatality rate among invasive meningococcal disease was 27.3% in this study.

3.1.2. Serogroup of N. meningitidis

Only serogroups B and C were identified among 11 invasive meningococcal disease patients. Serogroup B was present in majority (*n*=9) of patients and 2 serogroup C were found. All invasive meningococcal disease patients who had *N. meningitidis* serogroup C (*n*=2) succumbed.

3.1.3. Antimicrobial profile of N. meningitidis (n=8)

Penicillin was susceptible only in 2 (25.0%) patients with meningococcemia. The blood culture (4/6) isolates from patients with meningitis showed intermediate-susceptibility to penicillin. Susceptibility to ciprofloxacin (*n*=5), levofloxacin (*n*=5) and cotrimoxazole (*n*=7) was 62.5%, 62.5% and 87.5% respectively. Excellent susceptibility (*n*=8, 100.0%) was seen for cefotaxime and meropenem.

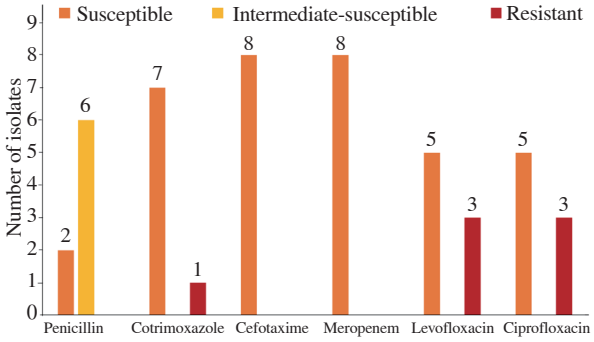

Figure 1. Antimicrobial susceptibility profile of *Neisseria meningitidis*.

3.1.4. Epidemiological link

Two distinct clusters with epidemiological links were observed in our study. One was in a prison and the other was linked to a religious place.

The prison cluster showed an epidemiological link to a prison inmate and a close relative of a prison inmate resided in the same prison, presented with invasive meningococcal disease during the same time period. Both were serogroup B with similar antibiogram showing resistance to ciprofloxacin.

There were two children (43 days old and 17 months old) with invasive meningococcal disease, residing in two different provinces,

presented at the same time period, linked epidemiologically to the same religious meeting place through their family contacts. These strains showed similar antibiogram and belonged to serogroup B.

3.2. S. pneumoniae

There were totally 26 *S. pneumoniae* clinical isolates, of which 20 isolates were from blood cultures and grouped as invasive pneumococcal diseases (IPD) including 4 from meningitis patients. The six non-invasive pneumococcal disease (n-IPD) isolates were from pus from eye (n=1), sputum (n=3), endotracheal secretions (n=1) and ear swab (n=1). There were 22 non-meningeal pneumococcal strains [IPD (n=16), n-IPD (n=6)] in this study group.

Majority patients were from hospitals in Western Province (n=15). Others were from Eastern (n=4), North Central (n=3), North Western (n=2) and Uva (n=2) provinces. Most were children aged 4 years old or below (n=12). The age ranged between 4 months to 67 years with male predominance (n=18, 69.2%).

3.2.1. Clinical information

Pneumonia (n=11) was the commonest clinical diagnosis in IPD. All IPD patient with serotype 3 (n=4) had pneumonia (Figure 2) while one isolate from n-IPD had otitis media.

In n-IPD, pneumonia or lower respiratory tract infections (n=4) were common. Other infections in n-IPD included dacryocystitis with an abscess and otitis media. All patients with serotype 6C (n=3) in both IPD and n-IPD had pneumonia.

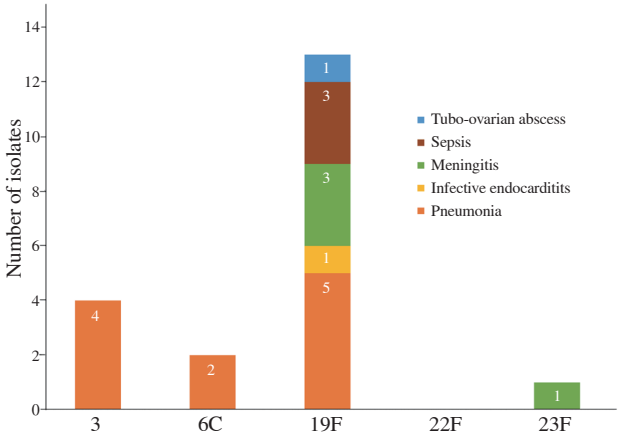

Figure 2. *Streptococcus pneumoniae* serotype distribution according to clinical syndrome in invasive pneumococcal disease.

3.2.2. Serotyping of S. pneumoniae and its antimicrobial profile

Serotype 19F was the most common serotype in IPD and n-IPD (Figure 3). MDR strains were present in all 5 serotypes. All serotype 3 isolates and 60.0% (9/15) of serotype 19F were MDR in IPD. Majority of the IPD (60.0%, 12/20) and n-IPD (83.3%, 5/6) pneumococcal strains were MDR (resistant to ≥3 classes of antibiotics). Resistance to erythromycin-clindamycin-tetracycline was 60.0% in IPD (12/20) and 66.7% (4/6) in n-IPD isolates. Erythromycin resistance is seen in majority of 19F strains in IPD (8/13) and all pneumococcal strains of serotype 3 (5/5) in both groups.

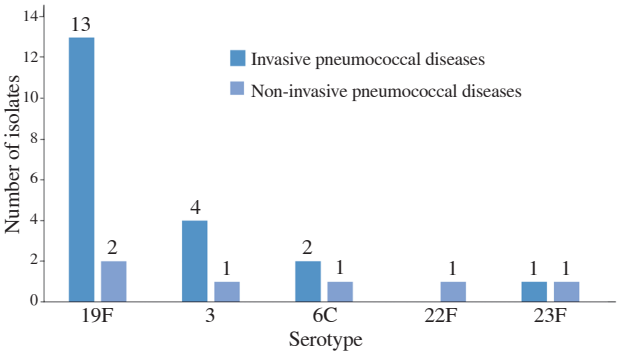

Figure 3. Serotype distribution of *Streptococcus pneumoniae* in invasive and non-invasive pneumococcal disease.

Pneumococcal conjugate vaccine 13 (PCV13) coverage for identified serotypes (19F, 3, 23F) is 60.0% (3/5) and 80.0% (4/5) for the pneumococcal polysaccharide vaccine 23 (PPSV23) in this study.

For *S. pneumoniae*, penicillin nonsusceptibility was interpreted as ≥0.12 µg/mL for meningitis strains and ≥4 µg/mL for non-meningeal strains.

Penicillin susceptibility in IPD was 70.0% (14/20), and non-susceptibility was 30.0% (6/20) (Figure 4). Penicillin non-susceptibility among all the non-meningeal strains were 13.6% (n=16, IPD and n=6, n-IPD). All four meningeal strains were penicillin-resistant and in addition, one strain showed intermediate susceptibility to cefotaxime. Amoxicillin was interpreted according to nonmeningitis breakpoints.

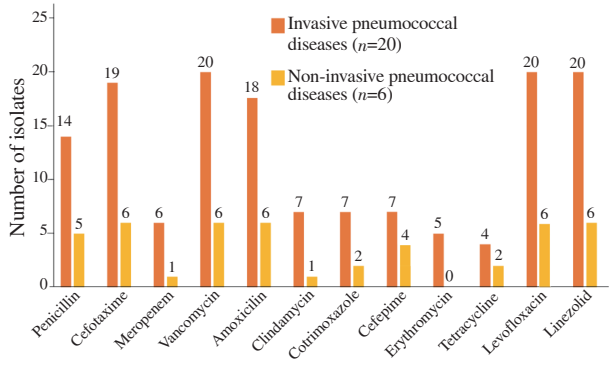

Figure 4. Susceptible *Streptococcus pneumoniae* in invasive and non-invasive pneumococcal diseases.

4. Discussion

Meningococcal and pneumococcal diseases cause invasive life-threatening infections in all age groups. Meningitis caused by these organisms is a public health challenge worldwide causing approximately 300 000 deaths and serious sequalae[5,12].

4.1. N. meningitidis

Serogroup B is common in many countries in Asia-Pacific region for invasive meningococcal disease and carriage[6]. India reported serogroup A as the predominant serogroup[13]. Serogroup B and C are the most prevalent serogroups in USA[14]. Similarly, we found serogroup B as the most common in our patients with few serogroup C isolates.

Even after introduction of antimicrobial therapy, current mortality of invasive meningococcal disease has remained at 10%-15% in the developed countries but higher in the developing world[3]. Studies revealed that bacteremia and meningococcal pneumonia alone have higher case fatality rate than meningococcal meningitis. Among the serogroups, case fatality rate has been highest in serogroup W and serogroup C[3]. In our study, the overall mortality rate was 27.3% and showed 100.0% for serogroup C. Meningococcemia and shock were the most common clinical manifestations seen in the 3 patients who died.

Penicillin was the first-choice antimicrobial for meningococcal infections until recent times. Majority (75.0%, 6/8) of our strains showed reduced susceptibility to penicillin. Transfer of mutations in penicillin-binding proteins within *Neisseria* species in the nasopharynx of colonized persons has been implicated as the leading cause for the decreased susceptibility to penicillin seen in meningococcus. High level penicillin resistance due to beta-lactamase production is rare[5].

Antimicrobial resistance in *N. meningitidis* is becoming well known and identified in other effective antimicrobials such as quinolones. A ciprofloxacin-resistant strain of serogroup C originally found in China has been identified from Canada and Japan[6]. Further, the emergence of ciprofloxacin-resistant, beta-lactamase producing *N. meningitidis* belonging to serogroup Y from USA is noteworthy[15]. We had three ciprofloxacin-resistant isolates. One strain showed ciprofloxacin-resistance with reduced susceptibility to penicillin belonging to serogroup B. Penicillin-resistant strains were not found in this study.

All our strains showed excellent susceptibility to cefotaxime and meropenem. Similar results were shown by Jorgenson *et al*[5]. However, reduced susceptibility to extended-spectrum cephalosporins had been reported from an outbreak in France[3]. Emergence of antimicrobial resistance in *N. meningitidis* highlights the importance of routine practice of antimicrobial susceptibility testing in microbiology laboratories.

There were two distinct clusters which were epidemiologically linked to a prison and a religious place observed in our study. Prisons are crowded, confined places where carriage rates of meningococci can be considerably high, creating a conducive environment for transmission[16]. Spill-overs can occur through visitors or prison inmates when released to the community after completion of their sentence. This could contribute to local and community outbreaks of drug-resistant *N. meningitidis*.

Furthermore, the three prison inmates in this study were from 3 different prisons. Two isolates from them belonged to serogroup B and one was serogroup C. We could retrieve only 2 isolates for AST. Their antibiograms differ by ciprofloxacin susceptibility result and belonged to serogroup B. This indicates the presence of multiple meningococcal strains in our prisons. Hence, we emphasize the need for meningococcal disease surveillance in prisons to prevent and control transmission of *N. meningitidis*.

Two children aged 43 days old and 17 months old residing in two different provinces were linked epidemiologically to the same religious place. They presented with invasive meningococcal disease at the same time period, acquired through their family contacts who had visited the same religious place. These two strains showed similar antibiogram and belonged to serogroup B. Asymptomatic carriage of meningococci by the adults of these two families could be explained for invasive meningococcal disease in these children.

Meningococcal colonization occurs in the nasopharynx between 5%-25% of the human population worldwide[3]. CDC meningococcal surveillance group analysis showed that household contacts has 500-800 times greater attack rates than the general population. Similar high-risk situations are present in closed institutions such as prisons, military barracks, long-term care hospitals[3].

Chemoprophylaxis is recommended to household or close contacts with invasive meningococcal disease patients. Close surveillance for secondary cases is essential which usually occur within 1-14 days[3]. In our study group, chemoprophylaxis was offered to all close contacts of the index cases including the prisons. Secondary cases were not reported to best of our knowledge.

4.2. S. pneumoniae

Different types of pneumococcal vaccines cover distinct serotypes[17]. Pneumococcal Polysaccharide Vaccine (PPSV) 23 has 80.0% coverage for the serotypes in this study. We had 3 pneumococcal isolates of serotype 6C, a non-vaccine strain usually found in countries after implementation of pneumococcal vaccines. Studies have shown that serotype 6A (a vaccine strain) has structural similarity and immunological cross-reactivity conferring partial

protection to serotype 6C diseases, unlike serotype 6B (another vaccine strain)[18]. In Sri Lanka, PPSV23 is given for high-risk adults. Absence of 6A in PPSV23 could pose a risk by an increase in 6C diseases, just as the strain 19A emerged worldwide after introduction of PCV7[19].

Pneumococcal conjugate vaccine 13 would give 60% protection in our study. Previous studies with Sri Lankan clinical isolates had shown 65% and 78.9% coverage for PCV13, respectively[19,20]. In both these studies, and our study, 19F was the predominant serotype. Further, introduction of PCV 13 would prevent most of the potentially fatal invasive pneumococcal diseases such as pneumonia, meningitis and sepsis caused mainly by serotype 3, 19F and 23F in our patients according to this study.

Emergence of antimicrobial resistance in pneumococcus is a major concern as treatment failure has become more common. MDR-strains are becoming more prevalent in Asian countries. Higher prevalence of MDR (59.3%) was found in Asian countries in comparison to North America (24%) and Europe (43%)[19].

In our study group, majority (60.0%) of *S. pneumoniae* strains were MDR. All serotype 3 isolates and majority of serotype 19F were MDR. National Reference Laboratory receive pneumococcal isolates from hospitals island wide for further testing with regard to identification and AST. This may have contributed to the higher percentage of MDR strains in this study.

Resistance to erythromycin-clindamycin-tetracycline was the most common MDR observed in this study. Macrolide-clindamycin-tetracycline phenotype is characterized by high-level erythromycin resistance, creating a higher risk of treatment failure in pneumococcal infections[21]. Serotype 19F and 6A have been implicated in carrying both *erm*B and *mef*A[19]. Erythromycin resistance is alarmingly high in our study, and majority belonged to serotypes 19F and 3. Similar results were shown in a previous study[19].

Batuwanthudawe *et al*. reported very high prevalence of penicillin nonsusceptibility (>90%) in IPD in Sri Lanka using <0.1 μg/mL as the breakpoint for penicillin[20]. Comparatively low penicillin nonsusceptibility in this study is due to the revision of Clinical Laboratory Standards Institute penicillin breakpoints in 2008. However, all patients with pneumococcal meningitis in this study were resistant to penicillin and belonged to serotype 19F (n=3) and 23F (n=1).

Higher rates of nonsusceptibility to oral anti-pneumococcal antimicrobials such as clindamycin, cefepime, erythromycin and meropenem in our isolates could be the result of their wider use in clinical practice. Studies have shown that high antimicrobial resistance is associated with the degree of antibiotic consumption in the community[22].

In contrast, pneumococcal isolates showed good susceptibilities to levofloxacin, linezolid, cefotaxime, amoxicillin and vancomycin. Further, amoxicillin with its good susceptibility profile can be a favorable choice of oral antimicrobial in non-invasive pneumococcal infections in our setting.

Immunization is the best preventive method for pneumococcal and meningococcal diseases to reduce morbidity and mortality. Nation-wide laboratory surveillance is necessary to determine the country-specific circulating serotypes of these virulent pathogens.

In conclusion, implementation of pneumococcal and meningococcal vaccines for children and high-risk population in the immunization program should be considered. It may reduce invasive diseases and prevent emergence of antimicrobial resistant strains. Pathogen-specific disease surveillance is a necessity in early recognition and prevention of outbreaks. Further, with introduction of vaccines, surveillance on country-specific serotypes would identify new or emerging serotypes early for necessary action.

Conflict of interest statement

All authors disclose no conflict of interest. No financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

Authors' contributions

LK and VB conceptualized and designed the study protocol; LK, VB, KDN, VR, AV, MD, VF, LY, CH, NS, PW, DN and TH carried out the work, laboratory examinations and interpretations, intellectual content, literature search, data acquisition; LK, VB, KDN, AV and VR performed data analysis and statistical methods; LK and VB drafted the manuscript. All authors read and approved the final manuscript.

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