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# Decreased *Streptococcus pneumoniae* susceptibility to oral antibiotics among children in rural Vietnam: a community study

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## Abstract

**Background:** *Streptococcus pneumoniae* is the most significant bacterial cause of community-acquired pneumonia among children under five years worldwide. Updated resistance information of *S. pneumoniae* among children is essential to adjust the recommendations for empirical treatment of community-acquired pneumonia, which will have immense implications for local and global health. This study investigated the prevalence of antibiotic resistance in isolated strains of *S. pneumoniae* and relationship with antibiotic use and demographic factors of children under five in rural Vietnam in 2007.

**Methods:** In Bavi district, 847 children 6 to 60 months were selected from 847 households. The main child-caregivers in the households were interviewed weekly using structured questionnaires to collect information of daily illness symptoms and drug use for the selected child over a four-week period (from March through June 2007). In the 3<sup>rd</sup> week, the children were invited for a clinical examination and to collect nasopharyngeal samples for *S. pneumoniae* identification. Etest and disk diffusion were used to test antibiotic susceptibility.

**Results:** Of 818 participating children, 258 (32%) had ongoing respiratory infections, 421 (52%) carried *S. pneumoniae*, and 477 (58%) had used antibiotics within the previous three weeks. Of the 421 isolates, 95% were resistant to at least one antibiotic (401/421). Resistance to co-trimoxazole, tetracycline, phenoxymethylpenicillin, erythromycin and ciprofloxacin was 78%, 75%, 75%, 70% and 28%, respectively. Low resistance was noted for amoxicillin (4%), benzylpenicillin (4%), and cefotaxime (2%). The intermediate resistance to amoxicillin was 32%. Multidrug-resistance was seen in 60%. The most common pattern was co-resistance to co-trimoxazole, tetracycline and erythromycin. The proportion of children carrying resistant bacteria was higher among the children who had used antibiotics in the previous three weeks.

**Conclusions:** Resistance to commonly used antibiotics and multidrug-resistance of *S. pneumoniae* in the area is remarkably high. High-dose amoxicillin is the only investigated oral antibiotic that can possibly be used for treatment of community-acquired pneumococcal infections. Strategies to promote appropriate prescribing and dispensing of effective antibiotics should be immediately implemented for the benefit of local and global health.

## Background

*Streptococcus pneumoniae* is the most significant bacterial cause of community-acquired pneumonia among children under five years worldwide [1]. *S. pneumoniae* often colonizes the nasopharynx of healthy children and can then, in specific situations, spread to the lungs,

paranasal tissues and cause mucosal infections, such as pneumonia, or invade the bloodstream and cause meningitis [2]. The infections caused by this pathogen are among those least likely to be resolved without effective antibiotic treatment. However, since 1980s, a dramatic increase in antibiotic resistance among *S. pneumoniae* has been observed in many parts of the world [3-7]. Treatment failure associated with antibiotic-resistant pneumococci has been reported for patients

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with pneumonia [7,8]. High level of antibiotic use is probably the main factor driving the emergence of resistance [9].

As in many other countries, a high prevalence of *S. pneumoniae* resistant to common antibiotics has been seen in studies in Vietnam [10,11]. A study conducted in Bavi district in 1999 showed that 90% of 106 *S. pneumoniae* isolates in 200 children were resistant to at least one antibiotic, 88% to tetracycline, 32% to trimethoprim/sulphonamide and 25% to chloramphenicol [10]. Updated resistance information, especially the resistance of *S. pneumoniae* among children, is essential to adjust the recommendations for empirical treatment of community-acquired pneumonia, which will have immense implications for local and global health.

This study is part of a larger project conducted among the population of a rural area in northern Vietnam where data on health care providers and consumers' knowledge as well as their use of antibiotics has been obtained [12,13]. The aim of this paper is to estimate the prevalence of *S. pneumoniae* carriage, to assess its antibiotic susceptibility, and to investigate possible associations to antibiotic use, demographic factors of children aged 6 to 60 months and households' economic status.

## Methods

### Study area

The study setting was Bavi district, 60 km west of Hanoi, the capital of Vietnam. The district is divided into lowland, highland and mountains. The population is approximately 262,000 persons, of whom 8% are children under five years of age. The basic public health care system includes a district hospital with 150 beds, 3 regional polyclinics and 32 commune health stations. An Epidemiological Field Laboratory (Filabavi) was established in 1998 [14]. Baseline and re-census surveys have been done every second year. Regular follow-up of vital events is conducted every three months. The surveillance database includes 69 clusters with about 51,000 inhabitants in 12,000 households. A cluster was defined as an administrative unit, usually a village. On average, there are about 600-700 inhabitants in each cluster.

### Subject and sample size

A sample of 847 children born from June 2002 to October 2006 was obtained from 847 households with at least one child eligible for the study. The sample size calculation was based on the assumption that antibiotic use in children under five in the community during one month was 70% [10], the expected drop out 30% and the design effect 2.0 due to clustering. Children older than 6 months were included to facilitate taking

nasopharyngeal samples. All the households in the largest clusters in each of three geographical areas were selected to give about 280 households in each area. Some clusters were excluded due to uneven economical distribution in quintiles. In each household, one child aged 6-60 months was randomly selected (if more than one such child was present). Participants' characteristics, date of birth, sex, residential areas, parents' education, and households' assets, expenditure, and income were extracted from *FilaBavi's* re-census survey 2007.

### Drug use survey and identification of *S. pneumoniae* carrier

The main child-caregivers in the households were interviewed weekly using structured questionnaires to collect information of daily illness symptoms and drug use for the selected child over a four-week period (from March through June 2007). The caregivers were also requested to fill a form regarding daily drug use for their child. When available, the drug package or prescription was examined by the interviewers. Drugs use for the participating children were classified according to the Anatomical Therapeutic Chemical (ATC) classification system [15], with the help of VN-pharmacy software [16]. This study includes antibiotics that are classified as antibacterials for systemic use and aggregated at the level of the active ingredient (level 5 of the ATC class J01) [15].

On the third Saturday of the survey period, the children were invited to the health commune station for a clinical examination by paediatricians from district or central hospitals. Trained microbiologists collected nasopharyngeal samples. The swabs were immediately placed in a transport tube with charcoal transport medium. Specimens were transported to Clinical Laboratories of National Institute of Infectious and Tropical Diseases, Hanoi, within 12 hours after sampling.

*S. pneumoniae* was isolated and identified using standard laboratory procedures [2,17]. Briefly, presumptive *S. pneumoniae* isolates were picked based on typical colony morphology,  $\alpha$ -hemolysis, and Gram stain morphology. Identification was confirmed by optochin sensitivity with an inhibition zone diameter of  $\geq 14$  mm. A single colony of each target bacterium was selected and sub-cultured for purity check and further diagnostic measures of *S. pneumoniae*.

### Antibacterial susceptibility testing

For all isolates, minimum inhibitory concentrations (MICs) were determined for benzylpenicillin and cefotaxime using Etest (AB bioMérieux, Solna, Sweden, formerly AB BIODISK). Inhibitory zone diameters were estimated for erythromycin, co-trimoxazole, tetracycline, and ciprofloxacin using disk diffusion (BIO-RAD Laboratories, Marnes-la-Coquette, France). The tested

antibiotics were those commonly used in the empirical treatment of pneumonia in Vietnam or recommended for testing by the Clinical and Laboratory Standard Institute (CLSI) [18]. Susceptibility testing was done according to the performance standards of CLSI [18] and manufacturers' instructions. *S. pneumoniae* ATCC 49619 were included as a control strain.

Interpretative non-meningitis breakpoints were based on the CLSI standards [18]. The inhibitory zone diameters for isolates to be considered resistant were: tetracycline  $\leq 18$  mm, co-trimoxazole  $\leq 15$  mm, erythromycin  $\leq 15$  mm. For ciprofloxacin, the criteria were: resistant  $\leq 15$  mm, susceptible  $\geq 30$  mm [19]. MICs values for cefotaxime in the Etest were: resistant  $\geq 4$  mg/l, susceptible  $\leq 1.0$  mg/l.

According to CLSI guidelines, the isolates with MICs  $\leq 2.0$  mg/l for benzylpenicillin were considered as susceptible at doses of at least 2 million units every four hours [18]. However, the possibility to administer the doses required by CLSI is commonly not feasible in primary health care. In order to translate *in vitro* resistance for benzylpenicillin to feasible treatment recommended for patients with pneumococcal pneumonia, we modified the current CLSI breakpoints using the European Committee on Antimicrobial Susceptibility testing (EUCAST) categorization as: susceptible MICs  $\leq 0.5$  mg/l, intermediate  $1.0$  mg/l  $\leq$  MICs  $\leq 4.0$  mg/l, resistant MICs  $\geq 8.0$  mg/l [18,20]. The pneumococcal infections caused by intermediate isolates may be appropriately treated with a high dose of antibiotics [18,20].

We defined the isolates as susceptible to amoxicillin and ampicillin using the same breakpoints as for benzylpenicillin [18]. Resistance to phenoxymethylpenicillin was derived from benzylpenicillin MICs  $> 0.06$  mg/l [20]. Multidrug resistance was defined as isolates resistant to at least three of six tested antibiotics, i.e benzylpenicillin, cefotaxime, erythromycin, co-trimoxazole, tetracycline, and ciprofloxacin. Benzylpenicillin, ampicillin, amoxicillin were grouped together as a single class. We did not include phenoxymethylpenicillin in the multidrug-resistant analysis since its susceptibility was derived from benzylpenicillin MIC.

#### Statistical analysis

Percentages of pathogen carriers and antibiotic-resistance of *S. pneumoniae* with 95% confidence interval (95% CI) were calculated to describe the nasopharyngeal carriage of bacteria among children and resistance among identified bacterium.

A wealth index defined as a combination of the information on household income, expenditure and assets was used to define the households' economic status [21]. The analysis based on this variable was initially done using quintiles. These were then dichotomized

into two levels: those identified as living below average conditions and those living at average or above conditions.

Multiple regression models were used to examine the statistical associations between antibiotic resistance and the independent variables: sex (male *vs.* female), age (24-60 months *vs.* 6-23 months), household's economic-status (average or above *vs.* below average), region (highland, mountains *vs.* lowland), present ARI symptoms including any signs of respiratory infections at the time of the nasopharynx sampling (yes *vs.* no), and antibiotics use within the three weeks preceding the sample collection (yes *vs.* no).

#### Ethical considerations

The ethical review board of Hanoi Medical University approved this study (No 28/HMURB, 2006). Verbal consent of all parents was sought after explanation of the purpose of the study. Confidentiality was assured and participants were informed of their right to withdraw at any time without any explanation. Children having a condition in need of medical treatment were treated and counselled by paediatricians from district or central hospitals.

#### Results

##### Study population and *S. pneumoniae* carriage

For the 847 selected children, 823 (97%) caregivers gave consent to participate in the drug use survey. Of these, 818 (99%) also consented for their child to participate in the clinical examination and collection of nasopharyngeal samples. The characteristics and current health situation of the children is presented in Table 1.

In the clinical examination, ARI symptoms were reported in 258 children (32%). The most common symptoms were mild ARIs such as cough, sore throat, stuffy nose, or runny nose. Seven children had fever  $> 38^{\circ}$  C. Only one child was diagnosed as having pneumonia. Children aged 6-23 months were more likely to present ARI symptoms than children aged 24-60 months (34% *vs.* 29%). There were no significant differences regarding the presence of ARI symptoms between geographical areas or households' economic conditions. Non-ARI symptoms such as digestive disorder, skin rash, toothache, phymosis were reported for 20% of the children.

Use of antibiotics during the three weeks preceding the nasopharyngeal collection was reported for 58% of the children. The average antibiotic course was 3.54 days (SD: 2.7 days), median 3.0 days indicating that 42% used antibiotics in short courses (one or two days) and a few reported a long treatment time. Penicillins with extended-spectrum (ATC code: J01CA) such as ampicillin, amoxicillin were most commonly used (49%), followed by cephalosporins (J01D) 27%, sulphonamids and

**Table 1 Characteristics of 818 community children aged 6-60 months and estimated prevalence of *S. pneumoniae* nasopharyngeal carriage.**

| Characteristics                        |                  | Absolute number with percentage in bracket |  |
|--|------------------|--|--|
|  |                  | Participating children<br>(% among total)  | <i>S. pneumoniae</i> carrier<br>(% within group) |
| Sex                                    | Female           | 373 (46)                                   | 187 (50)   |
|  | Male             | 445 (54)                                   | 234 (53)   |
| Age                                    | 6-23 months      | 367 (45)                                   | 203 (55)*  |
|  | 24-60 months     | 451 (55)                                   | 218 (48)*  |
| Region                                 | Lowland          | 268 (32)                                   | 145 (54)   |
|  | Highland         | 275 (34)                                   | 146 (53)   |
|  | Mountains        | 275 (34)                                   | 130 (47)   |
| Households' economic status            | Under average    | 358 (44)                                   | 196 (55)   |
|  | Average or upper | 460 (56)                                   | 225 (49)   |
| ARI symptoms at the sampling time      | No presence      | 560 (68)                                   | 265 (47)*  |
|  | Presence         | 258 (32)                                   | 156 (61)*  |
| Other observation <sup>§</sup>         | No presence      | 650 (80)                                   | 340 (52)   |
|  | Presence         | 168 (20)                                   | 81 (48)  |
| Previous antibiotic use within 3 weeks | No               | 341 (42)                                   | 183 (54)   |
|  | Yes              | 477 (58)                                   | 238 (50)   |
|  | Total            | 818 (100)                                  | 421 (52)   |

\* Significant different between groups using  $\chi^2$  test ( $p < 0.05$ ).

<sup>§</sup> Other observations: Non-ARI symptoms such as digestive disorder, skin rash, toothache, phymosis.

trimethoprim (J01E) 11%, macrolids (J01F) 10% and other antibiotics including tetracyclines (J01A) and aminoglycosids (J01G) 3%. Most of the antibiotic courses were used when nothing more than symptoms of mild ARIs were presented. Children living in the mountainous area more often used co-trimoxazole and those in the highland area more often used amoxicillin than other areas.

*S. pneumoniae* was isolated from 421 (52%) of the children. Children aged 6-23 months were more likely to carry *S. pneumoniae* than those aged 24-60 months (Table 1). Moreover, children who had ARI symptoms were more likely to carry the bacterium. There was no significant difference in carrier prevalence related to prior use of antibiotics or household's economic condition.

#### ***S. pneumoniae* antibiotic susceptibility**

Table 2 shows the in-vitro activity of nine antibiotics against 421 isolates of *S. pneumoniae*. Ninety-five percent of isolates (401/421) were resistant to at least one of investigated antibiotics. A high level of resistance was found to co-trimoxazole, tetracycline, phenoxymethylpenicillin and erythromycin (70-78%). Low resistance was noted for benzylpenicillin (or amoxicillin and ampicillin) (4%) and cefotaxime (2%). However, the intermediate resistance to benzylpenicillin (or amoxicillin and ampicillin) was 32%. More than one-quarter of the *S. pneumoniae* isolates demonstrated resistance to ciprofloxacin. Only one isolate was susceptible to all antibiotics.

The distribution of benzylpenicillin and cefotaxime MICs in the Etest is shown in Figure 1. The MIC of benzylpenicillin at which 90% of isolates was inhibited (MIC<sub>90</sub>) was 1.5 mg/l, while cefotaxime MIC<sub>90</sub> was 0.75 mg/l. Moreover, we found 12 and 7 isolates for which benzylpenicillin and cefotaxime MICs were higher than 32 mg/l (Figure 1.a, b). Almost all of those isolates were resistant to co-trimoxazole, tetracycline, erythromycin, and intermediate resistant to ciprofloxacin. In the disk diffusion tests, 200/421 and 250/421 strains showed no inhibitory zone (6 mm inhibitory-zone diameter) to co-trimoxazole and erythromycin, i.e. these antibiotics had no effect on most of the isolates (Figure 2.b, c).

Table 3 shows the relation between sex, age, economic status, geographic condition, previous antibiotic use and the prevalence of antibiotic resistance. Children living in the mountainous area were more likely to carry erythromycin and cotrimoxazol-resistant isolates than those in other areas. The proportion of children carrying resistant bacteria was higher among the children who had used antibiotics within the three weeks prior to the study for all antibiotics investigated. Prior treatment of co-trimoxazole gave a higher risk of cotrimoxazol resistance of the isolates.

#### **Multidrug resistance among *S. pneumoniae* isolates**

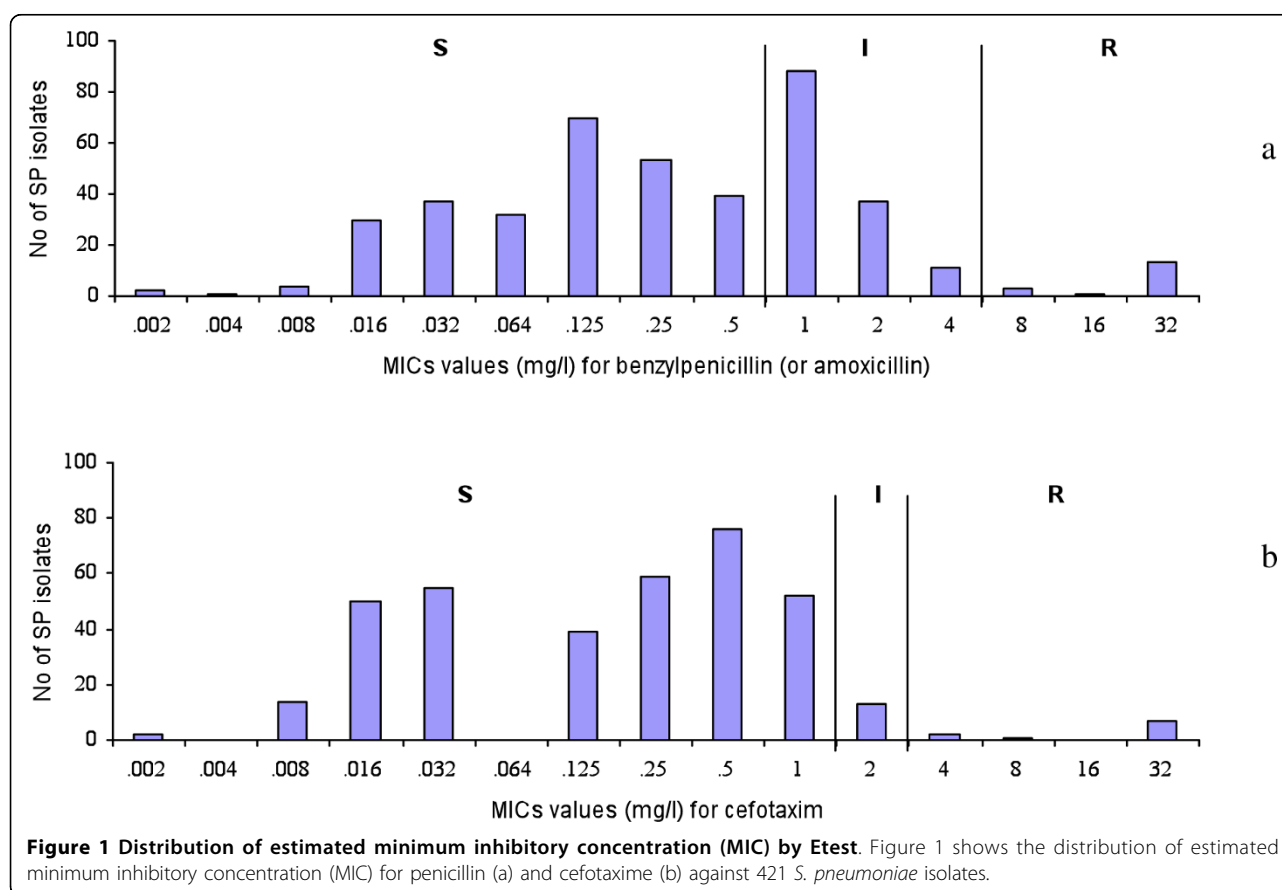
Most resistant isolates were multidrug resistant (252/401), and they accounted for 60% of all isolates. Isolates that were resistant to three classes of antibiotics were

**Table 2 Susceptibility of 421 *S. pneumoniae* isolates to antibiotic agents**

| Antibiotic agents       | ATC code | Absolute number and percentage in bracket |                  |                 |
|-------------------------|----------|---|------------------|-----------------|
|                         |          | Resistant (R)                             | Intermediate (I) | Susceptible (S) |
| Tetracycline            | J01AA07  | 314 (75)                                  | 44 (10)          | 63 (15)         |
| Benzylpenicillin        | J01CE01  | 17 (4)                                    | 136 (32)         | 268 (64)        |
| Phenoxyethylpenicillin* | J01CE02  | 315 (75)                                  | -                | 106 (25)        |
| Ampicillin**            | J01CA01  | 17 (4)                                    | 136 (32)         | 268 (64)        |
| Amoxicillin**           | J01CA04  | 17 (4)                                    | 136 (32)         | 268 (64)        |
| Cefotaxime              | J01DD01  | 9 (2)                                     | 14 (3)           | 398 (95)        |
| Co-trimoxazole          | J01EE01  | 329 (78)                                  | 47 (11)          | 45 (11)         |
| Erythromycin            | J01FA01  | 294 (70)                                  | 52 (12)          | 75 (18)         |
| Ciprofloxacin           | J01MA02  | 119 (28)                                  | 296 (70)         | 6 (2)           |

\* Susceptibility to phenoxyethylpenicillin is derived from benzylpenicillin MICs according to EUCAST documents.

\*\* Susceptibility to ampicillin and amoxicillin are derived from benzylpenicillin MICs according to CLSI documents.



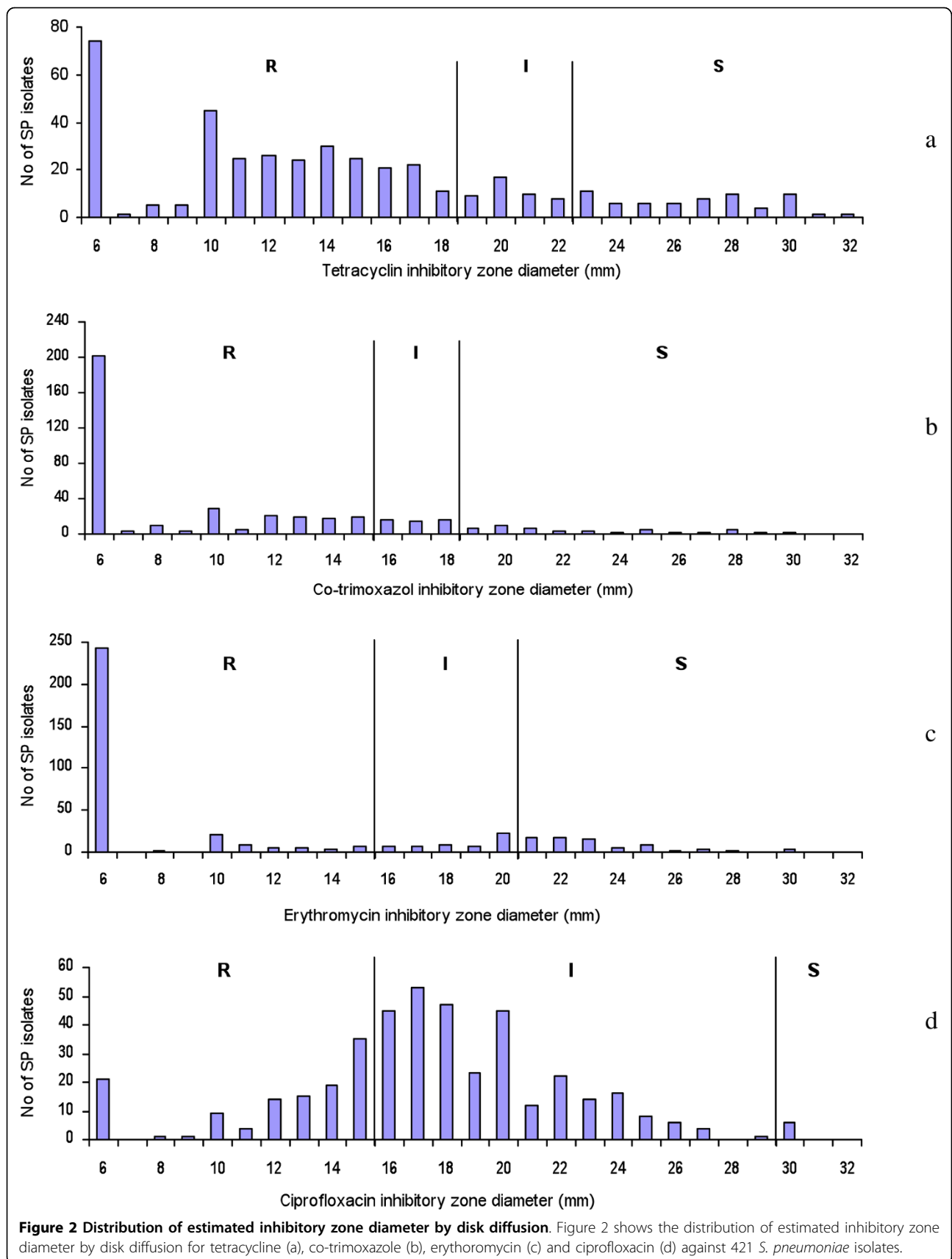
the most prevalent, 45% (190/421), then four antibiotics 14% (58/421). Co-resistance to five antibiotics was presented by 4 isolates.

Table 4 shows the pattern of co-resistance among the *S. pneumoniae* isolates. The most common pattern was co-resistance to co-trimoxazole, tetracycline, and erythromycin (200/252). Penicillin-resistant pneumococcal isolates are more likely to be concomitantly resistant to cefotaxime, and co-trimoxazole than to other antibiotics.

Multi-resistance was not associated with sex, age, or economic status but significantly higher in children living in the mountainous area compared to other areas (Table 3).

### Discussion

This study shows that almost all of the *S. pneumoniae* isolates from the children were resistant to at least one of the investigated antibiotics. Most of the children had



**Figure 2 Distribution of estimated inhibitory zone diameter by disk diffusion.** Figure 2 shows the distribution of estimated inhibitory zone diameter by disk diffusion for tetracycline (a), co-trimoxazole (b), erythromycin (c) and ciprofloxacin (d) against 421 *S. pneumoniae* isolates.

**Table 3 Antibiotic resistance in relation to background characteristics of the 421 *S. pneumoniae* carriers.**

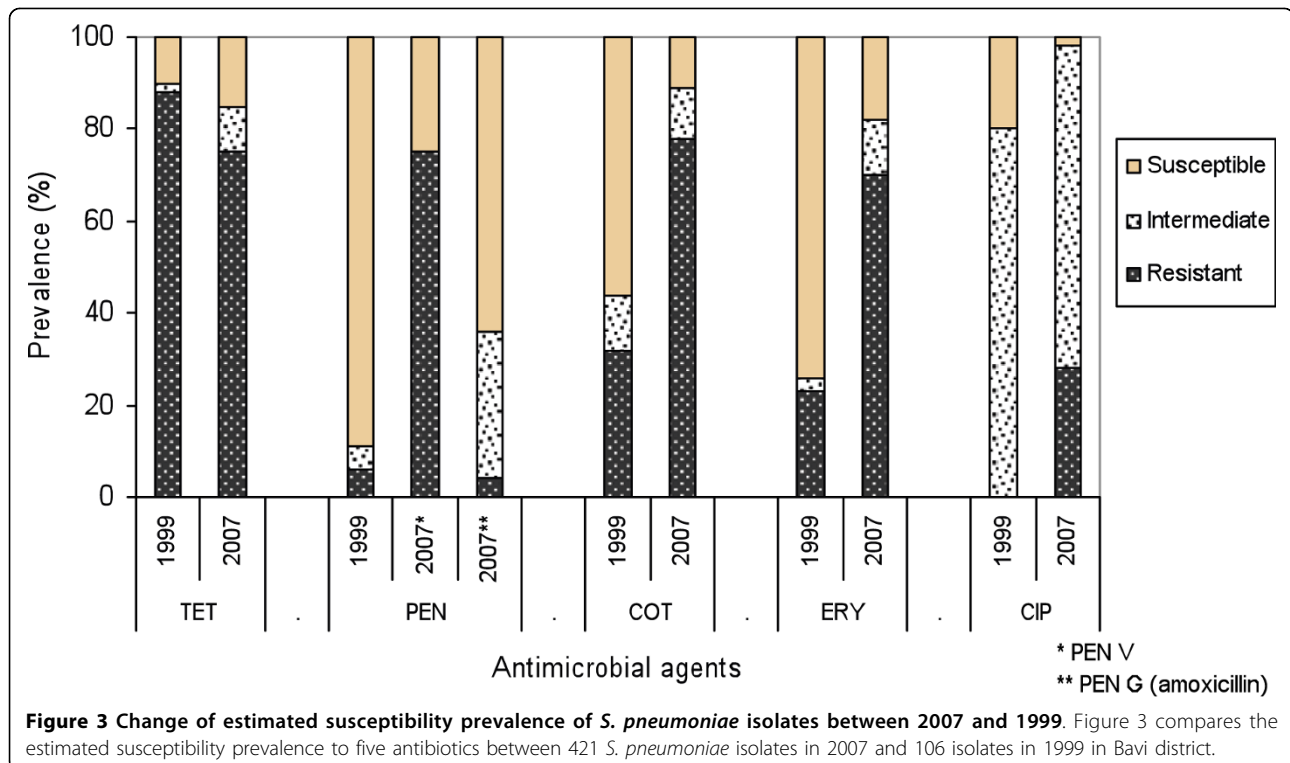
| Characteristics<br>Total number of carrier in bracket | Percentage of resistant strains among total carriers within each category |                                     |                    |                  |                  |                  |                  |                  |
|---|---|-------------------------------------|--------------------|------------------|------------------|------------------|------------------|------------------|
|   | TET <sup>†</sup>  | PEN G <sup>†</sup><br>(AMX,<br>AMP) | PEN V <sup>†</sup> | CTX <sup>†</sup> | SXT <sup>†</sup> | ERY <sup>†</sup> | CIP <sup>†</sup> | MDR <sup>†</sup> |
| <b>Sex</b>  |   |                                     |                    |                  |                  |                  |                  |                  |
| - Female <sup>R</sup> (187)                           | 68*   | 5                                   | 72                 | 3                | 78               | 67               | 25               | 56               |
| - Male (234)  | 80* (**)  | 3                                   | 77                 | 1                | 79               | 72               | 31               | 63               |
| <b>Age</b>  |   |                                     |                    |                  |                  |                  |                  |                  |
| - 6-23 months <sup>R</sup> (217)                      | 75  | 4                                   | 76                 | 3                | 78               | 73               | 27               | 61               |
| - 24-60 months (204)                                  | 74  | 2                                   | 74                 | 2                | 78               | 67               | 29               | 59               |
| <b>Region</b>   |   |                                     |                    |                  |                  |                  |                  |                  |
| - Lowland <sup>R</sup> (145)                          | 77  | 2                                   | 78                 | 0*               | 74*              | 68*              | 32               | 55*              |
| - Highland (146)                                      | 72  | 6                                   | 77                 | 5*               | 74*              | 64*              | 24               | 56*              |
| - Mountains (130)                                     | 75  | 4                                   | 69                 | 2*               | 88* (**)         | 78*              | 29               | 70* (**)         |
| <b>Economic status</b>                                |   |                                     |                    |                  |                  |                  |                  |                  |
| - Under average <sup>R</sup> (203)                    | 79  | 5                                   | 76                 | 3                | 80               | 72               | 31               | 61               |
| - Average or upper (218)                              | 71  | 3                                   | 74                 | 1                | 76               | 68               | 26               | 59               |
| <b>Prior antibiotic use</b>                           |   |                                     |                    |                  |                  |                  |                  |                  |
| - No <sup>R</sup> (183)                               | 73  | 3                                   | 68*                | 0.5*             | 73*              | 67               | 26               | 56               |
| - Yes (238)   | 76  | 5                                   | 80* (**)           | 3*               | 82* (**)         | 72               | 30               | 63               |
| Total (421)   | 75  | 4                                   | 75                 | 2                | 78               | 70               | 28               | 60               |

\* p < 0.05: significant different between groups using  $\chi^2$  test.

\*\* Significant difference between group vs. reference using multiple logistic regression analysis.

<sup>R</sup> Reference groups in multiple logistic regression analysis

<sup>†</sup> TET: tetracycline, PEN G: benzylpenicillin, AMX: amoxicillin, AMP: ampicillin, PEN V: phenoxymethylpenicillin, CTX: cefotaxime, SXT: co-trimoxazole, ERY: erythromycin, CIP: ciprofloxacin, MDR: multidrug-resistant.



**Figure 3 Change of estimated susceptibility prevalence of *S. pneumoniae* isolates between 2007 and 1999.** Figure 3 compares the estimated susceptibility prevalence to five antibiotics between 421 *S. pneumoniae* isolates in 2007 and 106 isolates in 1999 in Bavi district.

**Table 4 Pattern of co-resistance to tested antibiotics among 421 *S. pneumoniae* isolates**

| Individual antibiotic and combination | Absolute number of co-resistance with percentage of total 421 isolates in bracket |                    |                  |           |         |           |                 |  |
|---------------------------------------|---|--------------------|------------------|-----------|---------|-----------|-----------------|--|
|                                       | CIP <sup>†</sup>  | PEN G <sup>†</sup> | CTX <sup>†</sup> | CIP-PEN G | CIP-CTX | PEN G-CTX | CIP-CTX - PEN G |  |
| SXT <sup>†</sup>                      | 119 (28)  | 17 (4)             | 9 (2)            | 1 (0.2)*  | 2 (0.5) | 5 (1)*    | 0 (0)           |  |
| TET <sup>†</sup>                      | 329 (78)  | 86 (20)            | 17 (4)*          | 9 (2)     | 1 (0.2) | 2 (0.5)   | 5 (1)           |  |
| ERY <sup>†</sup>                      | 294 (70)  | 66 (16)*           | 15 (4)           | 6 (1)     | 1 (0.2) | 0 (0)     | 4 (1)           |  |
| SXT - TET                             | 254 (60)*   | 73 (17)            | 13 (3)           | 7 (2)     | 1 (0.2) | 2 (0.5)   | 3 (1)           |  |
| SXT - ERY                             | 248 (59)*   | 51 (12)            | 15 (4)           | 6 (1)     | 1 (0.2) | 0 (0)     | 4 (1)           |  |
| TET - ERY                             | 241 (57)*   | 60 (14)            | 13 (3)           | 5 (1)     | 1 (0.2) | 0 (0)     | 3 (1)           |  |
| SXT-TET-ERY                           | 200 (48)  | 45 (11)            | 13 (3)           | 5 (1)     | 1 (0.2) | 0 (0)     | 3 (1)           |  |

\*Significant different between resistant groups vs. non-resistant groups using  $\chi^2$  test ( $p < 0.05$ ).

<sup>†</sup> TET: tetracycline, PEN G: benzylpenicillin, CTX: cefotaxime, SXT: co-trimoxazole, ERY: erythromycin, CIP: ciprofloxacin.

used antibiotics during the three weeks preceding the sample collection. There was a statistical association between antibiotic use and resistance. All the oral antibiotics investigated showed very low level of susceptibility except for high-dose amoxicillin that can possibly be used for treatment of pneumococcal infections.

*S. pneumoniae* carriage prevalence estimate was 52%, similar to the results of some other studies in Vietnam [10,11], but higher than reported elsewhere [22,23]. The carriage was lower in the older children, which is in accordance with other studies [24]. Unlike previous reports [25], however, the association between nasopharyngeal carriage of *S. pneumoniae* and prior antibacterial use was not present and this might be explained by the high levels of resistance discovered. A relationship between pneumococcal nasopharyngeal carriage to the spread of the pathogen and the development of pneumococcal diseases has been reported [26]. Implementation of conjugate vaccine could protect against pneumococcal carriage and reduce the risk of developing infection [27,28]. However, these vaccines are still expensive and may not help to reduce nasopharyngeal carriage in non-vaccine serotype carriage [27].

The resistance to all oral commonly used antibiotics investigated among the 421 *S. pneumoniae* isolates was markedly higher than previous research [3-6]. The resistance prevalences were considerable higher than those reported in 1999 in the same area, except for tetracycline (Figure 3) [10]. A significant increase of drug-resistant pneumococcal isolates from rural area was also noted in Khanh Hoa province, Vietnam [29]. One of the most serious findings is the high multidrug resistance among the isolates (60%). This was distinctively higher than reported from the Netherlands, Russia and Sweden, however still lower than several other Asian countries [30,31]. The finding is particularly worrying, as co-resistance to co-trimoxazole-tetracycline-erythromycin is the most common pattern. The high prevalence of multi-

resistant pathogens shows that the practice of combining drugs in empirical treatment in the case of resistance might not be effective in this context. Nevertheless, the national IMCI guidelines recommend five days of antibiotic therapy (anyone of co-trimoxazole, oral amoxicillin, and erythromycin) for treatment of pneumonia among children 2 months up to 5 years in the community [32]. This study shows that, co-trimoxazole and erythromycin can be expected to be virtually useless in this context and the recommendation of these drugs should be seriously considered. The emergence of *S. pneumoniae* strains showing "very high" resistance to benzylpenicillin and cefotaxime has rarely been reported internationally [33]. To address the characteristics of such isolates requires further study. The phenomenon of high resistance to commonly used antibiotics would imply a challenge for empirical treatment of pneumococcal infections in the area.

Which factors could have led to the very high level of *S. pneumoniae* antibiotic resistance and multidrug-resistance in this region? One important factor is probably the frequent and irrational use of antibiotics that constitutes a constant selective pressure. In this study area, many children frequently use antibiotics [10,34]; antibiotic use was also higher among children with pneumococcal resistant strains. The higher level of co-trimoxazole and multidrug resistance in the mountainous area may mainly be due to higher use of co-trimoxazole in this area and leading to differential selective pressure in different areas. Some resistant clones of *S. pneumoniae* spreading with different degrees of success may also be responsible for the geographical difference of resistance [35]. The easy transmission of the pathogen between individuals could facilitate the spread of multidrug resistant strains through a small number of resistant mutants [30,36]. Previous studies found two pandemic clones Taiwan 19F and Spanish 23F to be important among pneumococcal isolates in Vietnam [3,11,28,29,37]. The global



spread of the pandemic multidrug-resistant serotypes possibly partly contributes to the increase in resistance in this region [37,38]. Further, the genetic determinants for resistant clones usually exist in a stable form, so once resistance is established, it is not easily lost [36]. Although the use of tetracycline in the region recently is very low compared to three decades ago, the persistence of high resistance to this antibiotic indicates the stability of the resistant clones [34]. High resistance to ciprofloxacin among the strains of children could be due to the spread of already resistant strains from adults [3,39]. The frequent inappropriate practices of prescribers and drug sellers regarding antibiotic use for treatment of acute respiratory infections may be one explanation for the high level of antibiotic use and resistance in the area [13]. Other explanation might involve patients' misperceptions and self-medication with antibiotics [12].

What antibiotics can possibly be used for empirical treatment of pneumococcal infections considering the high levels of antibiotic resistance? Clinicians may find it safe to choose susceptible antibiotics in empirical practice. On the other hand, if the effective antibiotics are more commonly used, this will promote the emergence of even more multidrug-resistant bacteria. Cefotaxime has the highest susceptibility among the investigated antibiotics. This drug is a broad-spectrum antibiotic and if used widely and indiscriminately, resistance to this antibiotic in *S. pneumoniae* may become a problem in the near future [28,40]. Hence, the still effective antibiotics need to be reserved for the severe cases in tertiary hospitals and in selected cases with troublesome resistance patterns. Benzylpenicillin has a low level of resistance (4%), but this antibiotic is administered by injections every 4-6 hours. Therefore, it is not feasible to use benzylpenicillin as the first-line for treatment. The use of narrow spectrum penicillins such as phenoxymethylpenicillin is higher among northern Europe countries where there is also generally a low rate of antibiotic resistance [9,41]. However, high resistance to phenoxymethylpenicillin in this study (75%) may lead to a poor clinical outcome if used as empirical treatment [8]. Amoxicillin is thus the only oral antibiotic that can possibly be used for treatment of pneumococcal pneumonia among children in the study area. Being low cost, easily accessible and efficacious, amoxicillin has been strongly recommended as the initial antibiotic for treatment of non-severe pneumonia especially where co-trimoxazole resistance is common [42]. According to pharmacokinetic-pharmacodynamic principles, the appropriate dose of amoxicillin is the one that maximizes the time when the plasma concentration persists above the MICs of the etiological agent ( $t > MIC$ ) [43]. For that reason, the aim is to increase the  $t > MIC$  by increasing the number of daily doses to achieve

bactericidal activity against isolates with  $MIC \geq 2$  mg/l [18,20,44]. To assure the therapeutic effect for at least 95% of pneumococcal infections in the context, the recommended dose of amoxicillin at 25 mg/kg  $\times$  3 times daily thus appears to be too low [32,42,44]. The guidance to increase the dose needs to be further demonstrated by clinical research.

The study has several strengths. It is a community study since the children were studied in their households and not in hospitals or clinics. The latter is a common approach but the selection process will mean that the studies only represent those seeking health care. The results are internally valid for those clusters actually investigated. The generalization of the result, the external validity, must be based on theoretical propositions suggesting that clusters or district are quite similar in respects of the phenomenon under study, the antibiotic resistance. Nothing indicates that similar rural areas should be very different. The clusters selected were the largest clusters for logistic reasons. As far as it can be investigated, nothing suggests a tendency to higher carriage or resistance in the larger clusters. The participation percentage is high. The study had been carefully piloted and field-tested. The training and supervising of the interviewers and lab workers had been conducted both in the field and in the laboratory to control the quality of collected data. By using both CLSI and EUCAST guidelines to define the interpretative breakpoints for benzylpenicillin, the resistance data is considered to be more useful for developing countries' conditions.

The major limitation of the study was that due to the limited resources, we only performed Etest for benzylpenicillin and cefotaxime. The susceptibility test for ciprofloxacin is not recommended by CLSI, but the test for *S. pneumoniae* is considered as an appropriate marker for monitoring fluoroquinolone resistance [19,20,45].

## Conclusions

Resistance to commonly used antibiotics and multidrug-resistance of *S. pneumoniae* in the investigated area are remarkably high. Of all oral antibiotics investigated, only high-dose amoxicillin can possibly be used as the first-line of choice for empirical treatment of pneumococcal infections among children in the area. The antibiotic use within the three weeks prior to the study was higher among the children with resistant pneumococci. In patients who truly warrant therapy, consideration must be given to use the most effective antibiotic class to achieve a favourable therapeutic outcome and prevent the emergence of antibiotic resistance. Strategies to promote appropriate prescribing and dispensing of effective antibiotics should be immediately implemented for the benefit of local and global health.

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### Authors' contributions

NQH, NVT, ML, BE, HDP, NTKC, CSL participated in the conception and design of the study, and revising paper critically for substantial intellectual content. NQH supervised the data collection, performed data analysis, and drafted the manuscript. NVT was responsible for laboratory testing. NVT, ML, BE, NTKC, CSL supported in the data collection and helped to draft manuscript. BE, HDP contributed to statistical analysis. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

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