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Nasopharyngeal carriage and antimicrobial susceptibility profiles of *Streptococcus pneumoniae* among children with pneumonia and healthy children in Padang, Indonesia

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Abstract

Streptococcus pneumoniae is one of the pathogenic bacteria causing invasive pneumococcal diseases such as pneumonia, sepsis, and meningitis, which are commonly reported in children and adults. In this study, we investigated the nasopharyngeal carriage rates, serotype distribution, and antimicrobial susceptibility profiles of *S. pneumoniae* among children with pneumonia and healthy children under 5 years old in Padang, West Sumatra, Indonesia. Nasopharyngeal swabs were collected from 65 hospitalized children with pneumonia in a referral hospital and from 65 healthy children at two day-care centers from 2018 to 2019. *S. pneumoniae* was identified by conventional and molecular methods. Antibiotic susceptibility was performed with the disc diffusion method. Out of 130 children, *S. pneumoniae* strains were carried by 53% and 9.2% in healthy children (35/65) and children with pneumonia (6/65), respectively. Serotype 19F was the most common serotype among the isolated strains (21%) followed by 6C (10%), 14, 34 (7% each), and 1, 23F, 6A, 6B (5% each). Moreover, 55% of the strains (23/42) were covered by the 13-valent pneumococcal conjugate vaccine. Most isolates were susceptible to vancomycin (100%), chloramphenicol (93%), clindamycin (76%), erythromycin (71%), and tetracycline (69%). Serotype 19F was commonly found as a multi-drug resistant strain.

DATA SUMMARY

Supplementary materials accompany this paper: File S1: Data Serotype and Antimicrobial Susceptibility Test.

INTRODUCTION

Pneumonia, an infection of the lung parenchyma, still remains a major cause of morbidity and mortality in children worldwide [1]. Pneumonia is the leading cause of death for children under 5 years of age that killed 740180 children (14% of all deaths of children under 5 years of ages) worldwide in 2019, occurring mainly in developing countries [2]. Pneumonia also results in one of the largest state expenditures both directly, through medical costs and indirectly, by the loss of working hours in the parents taking care of their sick children [3]. In Indonesia, 16% of children's deaths (aged between 29 days to 11 months) in 2019 were due to pneumonia followed by diarrhoea [4]. Meanwhile, pneumonia (9.5%) is the second largest cause of death in children between aged 12 to 60 months under five [4]. *Streptococcus pneumoniae*, as one of the causative agents of pneumonia, is an opportunistic pathogen colonizing the human nasopharynx [5]. This colonization can increase risk of infection depending on the immune system's condition [5, 6]. In this study, we investigated the nasopharyngeal carriage rates, serotype distribution, and antimicrobial susceptibility profiles of *S. pneumoniae* among children with pneumonia and healthy children under 5 years of age in Padang, West Sumatra, Indonesia.

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Keywords: children; community-acquired pneumonia; nasopharyngeal carriage; streptococcus pneumoniae.
Abbreviations: BAP, blood agar plate; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; MDR, multi-drug resistant; NP, nasopharyngeal; OXA, oxacillin; PCV13, 13-valent pneumococcal conjugate vaccine; SM-PCR, sequential multiplex PCR; STGG, skim milk-tryptone-glucose-glycerol; SXT, trimethoprim/sulfamethoxazole; TE, tris-EDTA; TET, tetracycline; UNT, untypeable; VAN, vancomycin.
A data sheet is available with the online version of this article.

METHODS

Study design and specimen collection

The study was conducted at the Dr. M. Djamil Hospital, a provincial referral hospital, and at two day-care centres located in Padang, West Sumatra, Indonesia. The children enrolled in the Dr. M. Djamil Hospital were admitted patients with clinical pneumonia from April 2018 to December 2019. The enrollment criteria of children with pneumonia included new or progressive infiltrates on chest radiographs with ≥2 of the following criteria: dyspnoea, cough, hemoptysis, chest pain, and fever occurring ≤14 days before admission. Healthy children under 5 years of age, who were attending day-care centres, were also enrolled from August to December 2019. Demographic characteristics and clinical data were collected and recorded in the case report form.

The Nasopharyngeal (NP) swab specimens were collected from both groups using flocked nylon swabs (Copan; Cat. No. 503CS01) placed into 1 ml skim milk-tryptone-glucose-glycerol (STGG) medium followed by vortex. Samples were then stored at −80 °C. The specimens were regularly shipped to the Eijkman Centre for Molecular Biology (Eijkman Research Centre for Molecular Biology), Jakarta with dry ice for the isolation and identification of *S. pneumoniae*.

Bacterial identification

The NP swab specimens were enriched by transferring 200 µl of swab-STGG medium into 6 ml enrichment media consisting of 5 ml Todd-Hewitt broth (BD Bacto; Cat. No. 249240) with 0.5% yeast extract (BD Bacto; Cat. No. 212750) and 1 ml rabbit serum (Gibco; Cat. No. 16120099), and then were incubated at 37 °C with 5% CO_2 for 5 h. A 10 µl of the enriched specimens was inoculated onto a sheep blood (8%) agar plate (sBAP, comprised of TSA II; BD cat. no. 212305, with 8% v/v sheep blood supplementation) and incubated at 37 °C with 5% CO_2 for 20 h [7]. After that, all blood agar plates were examined for suspected *S. pneumoniae* colonies with the following colony morphologies: alpha-hemolysis, mucoid, and depressed centre. Suspected colonies were streaked on a sBAP and a disc containing 5 µg of optochin was placed onto the inoculated media. Colonies susceptible to optochin (inhibition zone diameter >14 mm) and positive for bile solubility were identified as *S. pneumoniae*.

Bacterial DNA was extracted by enzymatic fast DNA extraction as follows: the overnight colony on BAP was resuspended in 300 µl of NaCl 0.85% and the suspension was vortexed. This mixture was then incubated at 70 °C for 15 min followed by centrifugation at 10000 r.p.m. for 2 min. The supernatant was discarded and the pellet was then resuspended with 50 µl Tris-EDTA (TE) buffer followed by homogenization. A volume of 8 µl hyaluronidase (30 mg ml^{-1}) and 12 µl mutanolysin (2500 U ml^{-1}) were added and the suspension was vortexed. The suspension was then incubated for 30 min at 37 °C followed by enzyme inactivation at 100 °C for 10 min. The mixture was then centrifuged at 10000 r.p.m. for 4 min and the supernatant containing the DNA was stored at −20 °C until further analysis. Serotype determination was performed by a sequential multiplex PCR (SM-PCR) as published by Carvalho *et al*. followed by Quellung reaction method as described previously [5]. The serogroup six results obtained by PCR was further tested by using enzymatic restriction digest to distinguish serotypes 6A, 6B, 6C, and 6D [8].

Antimicrobial susceptibility testing

All *S. pneumoniae* strains isolated from both groups were tested for antimicrobial susceptibility using the disc diffusion method according to the Clinical and Laboratory Standard Institute, 2019 [9]. In this study, seven antimicrobial discs (Oxoid) were used: erythromycin (15 µg), sulfamethoxazole-trimethoprim [co-trimoxazole] (1.25/23.75 µg), clindamycin (20 µg), chloramphenicol (30 µg), tetracycline, (30 µg), vancomycin (1 µg), and oxacillin (1 µg).

RESULTS

The NP swab specimens were collected from a total of 130 children; 65 hospitalized children with pneumonia (mean age of 11.2±10.5 months) during the one and half years period of study, and 65 healthy children (mean age of 29.4±16.1 months) that were recruited from daycares for a period of 4 months. In this study, the proportion of infants less than 1 year of age was 80.0% (52/65) for the admitted patients and only 13.8% (9/65) for healthy children. The children's characteristics from both groups are shown in Table 1. The majority of the hospitalized children's clinical symptoms were fever (72.3%), rhonchi (76.9%), and cough (66.2%). Most of the hospitalized children had history of previous antibiotics use (54/65; 83.1%) and chest X-ray infiltrates (56/65, 86.2%) (Table 1). The good nutritional status of healthy children was higher than children with pneumonia (72.3% vs 53.8% respectively).

In this study, we found that the nasopharyngeal carriage of *S. pneumoniae* was 9.2% (6/65) among the children with pneumonia and 53% (35/65) among the healthy children enrolled from the day-care centres. Among children with pneumonia, the *S. pneumoniae* carriage was different between age groups (Fig. 1). The carriage rates were 7.7, 20.0, and 12.5% for the age groups of less than 1 year of age (4/52), 13 to 24 months (1/5), and 25 to 60 months (1/8), respectively. Meanwhile, among healthy children,

Table 1. Baseline characteristics and bacterial *Streptococcus pneumoniae* findings among children with pneumonia and healthy children in Padang, West Sumatra, Indonesia

Variables		Children with pneumonia (*N*=65) n (%)	Healthy children (*N*=65), n (%)
Basic demographics			
Sex			
	Male	40 (61.5)	36 (55.4)
	Female	25 (38.5)	29 (44.6)
Age (month)			
	0–12	52 (80.0)	9 (13.8)
	13–24	5 (7.7)	24 (36.9)
	25–60	8 (12.3)	32 (49.2)
Exclusive breastfeeding			
	Yes	39 (60.0)	49 (75.4)
	No	22 (33.8)	16 (24.6)
Nutritional status			
	Malnutrition	29 (44,6)	18 (27.7)
	Good	35 (53.8)	47 (72.3)
Number of family member			
	1–3	No Data (ND)	33 (50.8)
	4–6	ND	30 (46.2)
	>7	ND	2 (3.1)
Smoking exposure			
	Yes	ND	45 (69.2)
	No	ND	20 (30.8)
Clinical symptom			
	Cough	43 (66.2)	ND
	Fever	47 (72.3)	ND
	Rhonchi	50 (76.9)	ND
	Chest X-ray infiltrates	56 (86.2)	ND
History of antibiotics			
	Yes	54 (83.1)	ND
	No	11 (16.9)	ND
Bacterial carriage in the nasopharynx			
Positive *S. pneumoniae*		6 (9.2)	35 (53.8)

ND, No Data.

the *S. pneumoniae* carriage rates were 55.6, 54.2, and 53.1% for the age groups of less than 1 year of age (5/9), 13 to 24 months (13/24), and 25 to 60 months (17/32), respectively (Fig. 1).

A total of 42 *S. pneumoniae* strains were isolated from 41 positive NP swab specimens, with a single sample (PDG 98) from one healthy-children simultaneously positive for strains of serotype 14 and 19A (Table S1). Serotype 19F was the most common serotype among the cultured strains (21%; 9/42) followed by 6C (10%; 4/42), 14, 34 (three carriers each;

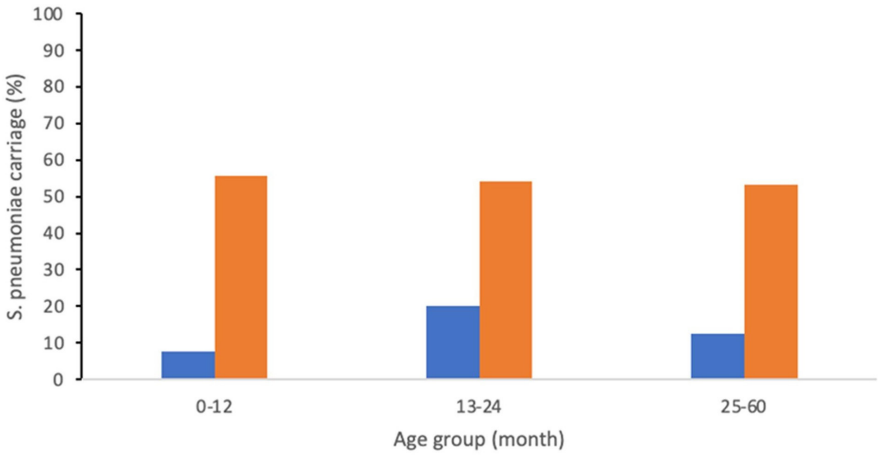

Fig. 1. Prevalence of *S. pneumoniae* carried by children with pneumonia (blue bar) and healthy children (orange bar) based on age groups: 0–12 months, 13–24 months, and 25–60 months.

7% each), 1, 23F, 6A, 6B (two carriers each; 5%), and 4, 11A/11D, 15B/15C, Serogroup 18, 19A, 33F/33A/37 (one carrier each, 2%) (Fig. 2a). We found that nine isolates (21%) were untypeable (UNT) strains using the SM-PCR method. Among children with pneumonia, we isolated six *S. pneumoniae* isolates including serotype 19F and 6B (one strain each) and four UNT strains, while among healthy children, we identified thirty-six *S. pneumoniae* isolates with serotype 19F as the most prevalent serotype among cultured strains (8/36) (Fig. 2b). In this study, *S. pneumoniae* strains that were covered by the pneumococcal conjugate vaccine (PCV13) was 50%.

We found that the majority of *S. pneumoniae* isolates were susceptible to vancomycin (100%), chloramphenicol (92.9%), clindamycin (76.2%), erythromycin (71.4%), tetracycline (69%), oxacillin (38.1%) and co-trimoxazole (28.6%) (Fig. 3a). Among *S. pneumoniae* isolated from children with pneumonia, we found that all isolates were resistant to oxacillin and co-trimoxazole (100%) followed by tetracycline (67%), erythromycin (50%) and clindamycin (33%) (Fig. 3b). Concordance with *S. pneumoniae* isolated from healthy children, we also found that the isolates were resistant to oxacillin and co-trimoxazole followed by tetracycline, erythromycin and clindamycin (Fig. 3c). Vancomycin is found susceptible for all of isolates from healthy children and children with pneumonia (Fig. 3b and Fig. 3c).

Furthermore, we found that there were 36% (13/36) and 67% (4/6) of *S. pneumoniae* grouped as multi-drug resistant (MDR) with resistance to ≥3 groups of antibiotics among isolates of *S. pneumoniae* isolated from healthy children and children with pneumonia respectively (Fig. 4a). The serotype 19F is the most common serotype found as MDR (Fig. 4b, c).

DISCUSSION

In this study, we found that the prevalence of *S. pneumoniae* in children with pneumonia (9.2%) was lower than healthy children (53%) in Padang, West Sumatra, Indonesia. A possible explanation for this was due to previous antibiotic use at the secondary level hospital before being referred to our third level hospital and majority of children with pneumonia symptoms are under 1 year old (80%). A previous study in Thailand also reported that children with pneumonia showed lower prevalence of *S. pneumoniae* colonization (54.5%) compared to community controls (62.5%) [10]. However, this is in contrast with a previous study in India reporting that the prevalence of *S. pneumoniae* carriage among children with clinical pneumonia was higher (74.7%) than community children (54.5%) [11]. In comparison with a previous study, the range of prevalence of pneumococcal carriage among children under 5 years of age in Indonesia was 13.9–68% [12]. In addition, a previous study reported that attendance of children in daycare showed significant impact on *S. pneumoniae* colonization [13]. Another study reported that incidence of pneumonia was higher in malnourished children and is significantly different in children with no malnutrition as previously reported [14]. Furthermore, malnutrition was reported to correlate with increase severity and fatality of pneumonia case incidence [1].

In this study, we discovered that the serotypes circulating in West Sumatra, Padang are dominated by the invasive serotypes currently covered in the pneumococcal vaccine. More than half of isolates are included in the pneumococcal vaccine conjugate. This is also in concordance with previous studies which reported the common serotypes of *S. pneumoniae* circulating in Indonesia are the vaccine serotypes [15–17]. The vaccine serotypes are common serotypes distributed in Indonesia because Indonesia has not included the pneumococcal vaccine as part of the national routine vaccination programme. Among the vaccine serotypes, this study found that serotype 19F is the most frequent serotype identified. The frequency of each serotype colonizing children's

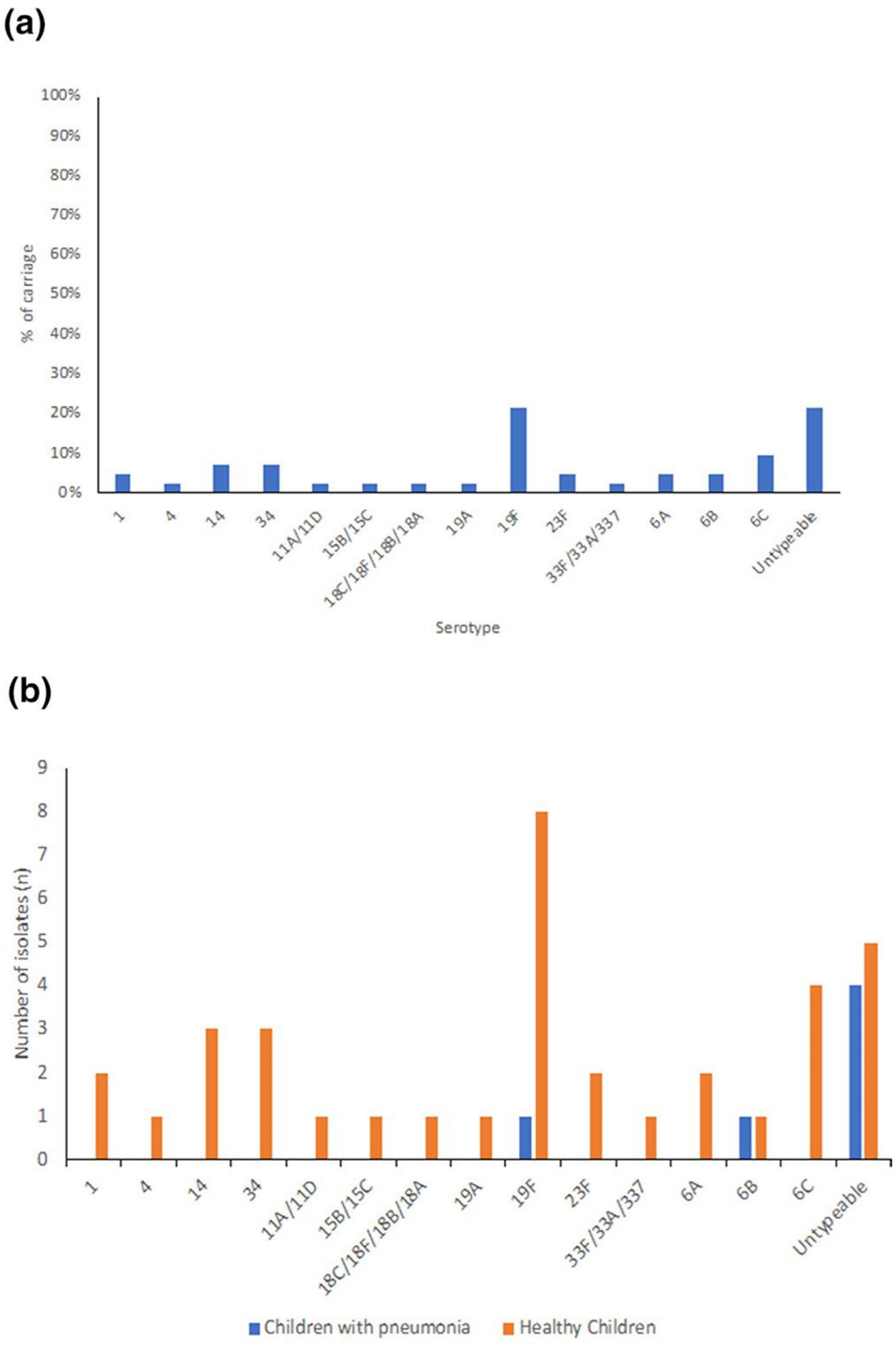

Fig. 2. Serotype distribution. (a) Among 42 *S. pneumoniae* carriage isolates of children with pneumonia and healthy children in Padang, West Sumatra, Indonesia, (b) serotype distribution of each group.

nasopharynx is found to vary across regions in Indonesia. In Kotabaru, Kalimantan and Lombok, West Nusa Tenggara, the most common serotype is 6A/6B [15, 17]. Meanwhile in Bandung, West Java, the 15B/15C is the most common serotype found in healthy children's nasopharynx.

This study also found that most of the isolates are resistant to co-trimoxazole and oxacillin which is also in concordance with recent publications reporting most of the *S. pneumoniae* isolates as being resistant to co-trimoxazole [17–19]. Moreover, we

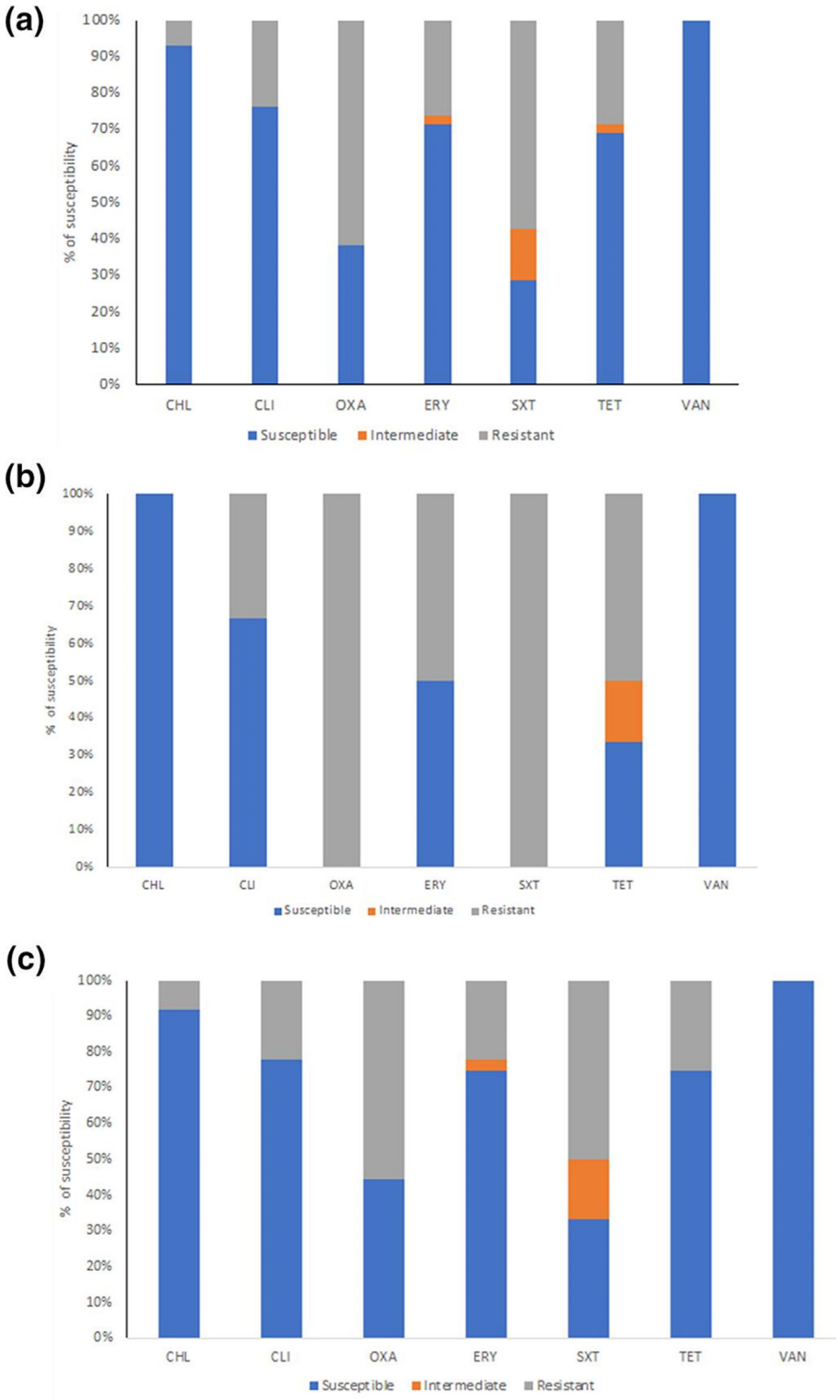

Fig. 3. Antimicrobial susceptibility profile of *S. pneumoniae* isolates in Padang, West Sumatra, Indonesia. (a). Antimicrobial susceptibility profile of total *S. pneumoniae* strains found in this study. (b) Antimicrobial susceptibility profile of *S. pneumoniae* strains isolated from children with pneumonia. (c). Antimicrobial susceptibility profile of *S. pneumoniae* strains isolated from healthy children. CHL: Chloramphenicol; CLI: Clindamycin; OXA: Oxacillin; ERY: Erythromycin; SXT: Trimethoprim/Sulfamethoxazole; TET: Tetracycline; and VAN: Vancomycin.

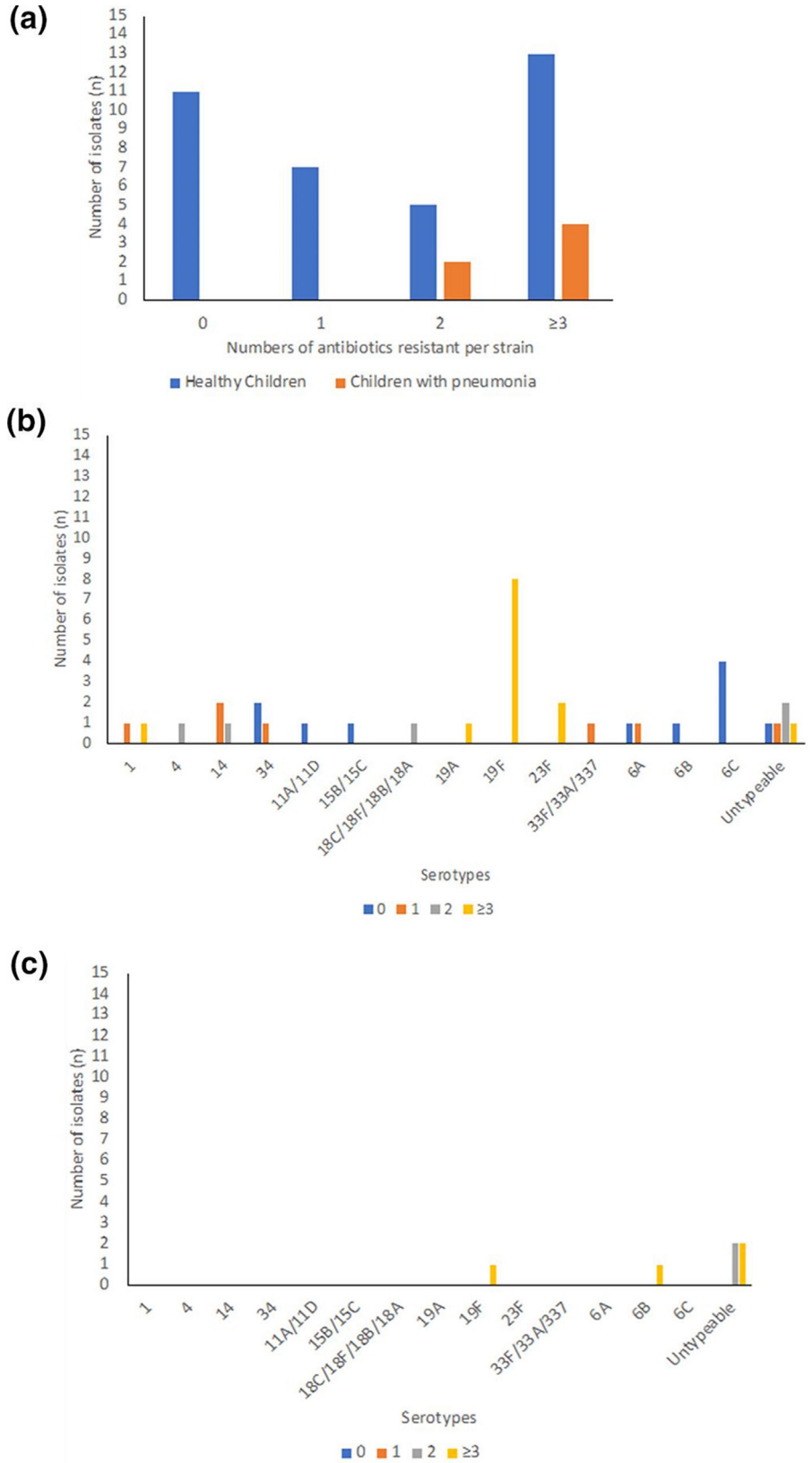

Fig. 4. The antimicrobial resistance of *S. pneumoniae* isolates. (a) Number of isolates with resistance number, (b) numbers of resistance serotypes of isolates from healthy children, (c) numbers of resistance serotypes of isolates from children with pneumonia.

discovered that not only is serotype 19F the most common serotype but is also resistant to more than three groups of antibiotics, identifying serotype 19F as MDR. This serotype has also been reported as the most common serotype with MDR characteristics in recent studies [17, 20].

The limitations of this study are related to the status of the hospital as the tertiary hospital and a referral centre, in which the history of antibiotic administration could not be provided for this study. In addition, in this study, the hospitalized children with pneumonia group was mostly children under 1 year of age, while in the healthy group there were more children over 1 year of age. This might affect the results of the carriage rates of *S. pneumoniae*. Children under 1 year of age are not common in day-care centres compared to children over 1 year of age, causing the healthy group to have varying results of colonization due to the high risk of pneumococcal transmission. In conclusion, prevalence of pneumococcal carriage in hospitalized children with pneumonia is lower than healthy children under 5 years old in Padang, Indonesia and serotype 19F was commonly found as a multi-drug resistant strain.

Funding information
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We thank to Microbiology Laboratory staff of the Dr. M. Djamil Hospital/Faculty of Medicine, Universitas Andalas, Padang, West Sumatra and the Molecular Bacteriology Unit Staff, the Eijkman Jakarta Institute of Molecular Biology, Jakarta who have helped in conducting this study.

Author contributions
F.F.Y.: chief investigator, study design, data collection, review draft manuscript. R.J.J.: coinvestigator, study design, data collection, data analysis, review draft manuscript. W.T.: coinvestigator, data collection, data analysis, preparation of manuscript. L.L.: data collection,review draft manuscript. I.H.: data collection, review draft manuscript. N.R.M.: data collection, review draft manuscript. D.S.: co-investigator, study design, data analysis, preparation of manuscript.

Conflicts of interest
The authors have declared that no competing interests exist.

Ethical statement
The study has been reviewed and approved by the ethical committee of Faculty of Medicine, Universitas Andalas, Padang, West Sumatra, Indonesia (No. 599/KEP/FK/2018, 618/KEP/FK/2019, and 649/KEP/FK/2018). Children were enrolled only after the parents or legal guardians signed an informed consent form.

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Peer review history

VERSION 2

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000584.v2.3

John Munnoch; University of Strathclyde, SIPBS, UNITED KINGDOM, Glasgow

Date report received: 15 May 2023
Recommendation: Accept

Comments: The work presented is clear and the arguments well formed. This study would be a valuable contribution to the existing literature. This is a study that would be of interest to the field and community. Thank you for addressing reviewer comments, I'm happy to recommend acceptance of the manuscript. Thank you again for choosing Access Microbiology and we welcome future submissions. Best wishes, John.

SciScore report

https://doi.org/10.1099/acmi.0.000584.v2.1

iThenticate report

https://doi.org/10.1099/acmi.0.000584.v2.2

Author response to reviewers to Version 1

Dear Academic Editor of the Access Microbiology Journal,

We thank the editor and reviewers for the critical assessment and suggestion of our manuscript (ACMI-D-23-00027) titled "Naso-pharyngeal carriage of Streptococcus pneumoniae strains and antimicrobial susceptibility carried by hospitalized pneumonia patients and healthy children under-five years old in Padang, Indonesia". The reviewers' comments were helpful in improving the overall quality of our manuscript. We have revised our manuscript according to the comments and suggestions made by the reviewers and editors. We thought that the issues raised were valuable and that it has led to a substantial improvement of the manuscript which we hope now is acceptable for publication in in the Access Microbiology Journal.

Regards,

On behalf of all authors

Dodi Safari

Rebuttal Letter

Reviewers' comments

Reviewer #1

Reviewer 1 Comments to Author:

This manuscript presents data analysing the carriage of *S.pneumoniae*in a cohort of healthy children vs those with pneumonia, from a tertiary care hospital in Indonesia. The study focuses on characterising the strains isolated from this cohort and their

resistance to anti-microbials. There are no apparent ethical or conflict-of-interest concerns, and the methods for the isolation, identification and analysis of the strains are sound. The results are displayed clearly, with some minor changes to graph formatting. Generally, the recommended edits relate to typographical issues and clarification to improve ease of understanding for the reader before publication.

Response: Thank you very much for the supportive feedback and suggestion. We have revised the manuscript according your input and suggestions.

37 - Change comma to decimal point.

Response: We revised it.

55 - 808,000 out of what, in what country?

Response: Thank you for your comment. We have revised according to the reference. It is 740,180 children under the age of 5 worldwide data reported by WHO as below:

"Pneumonia is the leading cause of death for children under 5 years of age, that killed 740,180 children (14% of all deaths of children under 5 years of ages) worldwide in 2019, and these deaths occur mainly in developing countries" (Line 56-58)

60 - 12% of the 16%? Or 12% of all total deaths

Response: The 12% is the percentage of diarrhea not for pneumonia, We removed the percentage of diarrhea (12%) to avoid misunderstanding (Line 61-64) .

60 - 9.5% of pneumonia? Or the 10.7%?

Response: We have removed the diarrhea data to avoid misunderstanding (Line 61-64).

62-64 – References

Response: we have added the references.

95 -Cat number but no supplier ref

Response: we have added the supplier reference.

95- 96 - in how much media? Should be percentages.

Response: It is clearly stated 1 ml of STGG. This STGG is transport broth media. There is no proportion of NP Swab and transport media should be added (Line 96-97).

101 - STGG abbreviation ? what is this?

Response: we have added in line 97-98

STGG = skim milk-tryptone-glucose-glycerol

102 - What is the enrichment media comprised of? what is Todd-Hewit broth comprised of?

Response: The enrichment broth is comprised of 5 ml Todd-Hewitt broth and 1 ml of Rabbit Serum as stated in line 106-107. The Todd-Hewitt broth is broth media commercialized in powder form. We have added the vendor information as well as the catalogue number of these reagents.

104 - What is the blood agar comprised of?

Response: Blood agar is comprised of Tryptic Soy Agar (TSA) II enriched with sheep blood for 8%. We have added this information in line 108-109.

108 - What is inoculated media comprised of?

Response: The inoculated media is sheep blood agar inoculated with enriched specimens.

113 - Decimal point instead of comma in percentages.

Response: We revised it.

115 - Replace decimal point with comma in percentages.

Response: We revised it.

116 - What is in the TE buffer?

Response:TE buffer is Tris-EDTA Buffer. We have added the information in the line 121.

120 - Make the rpm number the same as previous style.

Response: we have revised the rpm number (Line 125)

130-133 - These should be concentrations rather than weights.

Response: As stated in CLSI Guidelines, for disk diffusion the antibiotic amount used is in wight of antibiotics (in microgram) as well as in the insert package of antibiotic disk we used.

130 - Reference for the method.

Response: we have added the reference for the methods.

156 - Decimal point instead of comma at 12.5%.

Response: We revised it.

142 -150 - 131 patients mentioned in text versus 130 in table, extra patient is unaccounted for?

Table 1- Numbers don't always add up to 65 or the full cohort number, but no explanation provided.

Response: Thank you for your comments. We confirmed that number of participants = 130 children; children with pneumonia = 65 and healthy children = 65. We revised the text.

We found that one NP swab specimen (PDG 98) from one healthy-children positive simultaneously for strains of serotype 14 and 19A (Line 169-171)

Figure 1 - Y-axis should extend to 100% and axis line is missing. Y-axis label should be clearer e.g. % of children. No stats provided on the graphs, and blue bars e.g. hospitalised patients 13-24 months looks about double that of the other two age brackets -the results text mentions that there is a difference but no statistical analysis to back it up.

Response: Thank you for your suggestion. We modified the Figure 1 and the text as below

"The carriage rates were 7.7%, 20.0%, and 12.5% for the aged group of less than one year of age (4/52), the aged group of 13 to 24 months (1/5), and the aged group of 25 to 60 months (1/8), respectively. Meanwhile, among healthy children, the S. pneumoniae carriage were 55.6%, 54.2%, and 53.1% for for the aged group of less than one year of age (5/9), the aged group of 13 to 24 months (13/24), and the aged group of 25 to 60 months (17/32), respectively (Figure 1)." Line 161-167.

Figure 1 legend - S. pneumoniae

Response: We have revised it

Figure 2a - Y-axis should extend to 100%, no line for Y-axis, Y-axis label should be clearer.

Response: We have revised the Figure 2a. according to the suggestion.

Figure 2b - No line for Y-axis

Response: We have revised the Figure 2b. according to the suggestion.

Figure 3a - No line for Y-axis, units on Y-axis should have decimal point rather than comma, Y-axis needs label, different style of graph compared to figure 3b, 3c.

Response: We have revised the graph and use same graph style to present the results of three figures (figure 3a, figure 3b and figure 3c) according to your suggestions.

Figure 3b, Figure 3c - Y-axis needs label to clarify what percentages are.

Response:we have revised the Y-axis label as % of susceptibility includes susceptible, intermediate and resistant.

Figure 3 legend - Explain abbreviations or include abbreviations of antimicrobials in the results text, no explanation of what figure 3c shows.

Response: We have added information describing the abbreviation of antimicrobial used as well as the figure 3c information.

180 -182- confusing wording, state that the strains are less susceptible to these compounds rather than the compounds have been less susceptible.

Response: Yes, you are correct. The statement should describe the resistance profile of *S. pneumoniae*isolates against the compounds/antimicrobials.

186 - Explain what MDR is an abbreviation of.

Response: we have added the information in line 194

Figure4a - Might be helpful to state which antibiotics in figure legend, Y-axis has no line, Y-axis needs label. X-axis label could be clearer e.g. number of antibiotics resistant per strain.

Response: We have revised the graph as your suggestions. We have added the Y-axis label and revised the X-axis label.

188 - Change commonest to most common

Response:we have replaced the commonest to most common.

Figure 4b, 4c - Key should be clearer e.g. state that this is number of antibiotics in the graph, Y-axis has no line.

Response: we have revised the figure 4b and 4c according to your suggestions.

208 - Change comma in 54,5% to decimal point, same with 62,5%

Response: We have revised the comma to decimal.

212 - Change comma in 13,9 to decimal point

Response: We have revised the comma to decimal.

223-224 - Clarify that this is a test to diagnose pneumonia and the 90.3% was correct identification

Response: Correct, the chest X-ray is used to diagnose pneumonia.

234 - Change to "is found to vary" rather than is found vary

Response: We have revised as your suggestion.

235 + 236 + 244- Change commonest to most common.

Response: We have changed the commonest to the most common.

252- Ampicillin and gentamycin is mentioned but previous history of antibiotic administration could not be provided. clarify that this is inferred based on common treatment routine.

Response: Thank you for your suggestion. We removed it.

309-310 - Reference 4 style is not consistent with other references

Response: We revised it.

248-250 - Children under 1 year of age less likely to be in day-care? Which may explain some results e.g. older children more likely to be exposed to *S. pneumoniae*.

Response: This might explain the different rate of colonization between 2 groups. We have added your suggestion into manuscript line 249-252.

Reviewer #2

Reviewer 2 Comments to Author: In this manuscript, the authors set out to address a lack of information on the carriage of Streptococcus pneumoniae, a causative agent of pneumonia, in healthy and hospitalized children in Indonesia. They also attempt to identify the serotypes circulating in these cohorts and test their susceptibility to antimicrobials.

I have no major concerns about the manuscript - the methods are well articulated and the authors have done what they set out what they attempted to do, namely: describe the prevalence, serotype frequency, and antimicrobial susceptibilities of S.pneumoniae in cohorts of healthy and hospitalised children in the Padang region of Indonesia. They ultimately find that few hospitalised children actually carry S.pneumoniae in the nasopharynx, identify the most common serotype of S.pneumoniae found in the these cohorts, and provide support for a recent study suggesting that S.pneumoniae is commonly resistant to a commonly used antimicrobial (co-trimoxazole). There are significant age differences between the hospitalized and healthy cohorts that could account for differences in S.pneumoniae prevalence but the authors acknowledge and discuss this in the manuscript. They also discuss their findings in relation to other studies reporting S.pneumoniae carriage and antimicrobial susceptibility in the wider region.

I only have a few minor comments, most of which concern the formatting and presentation of the manuscripts figures. While the manuscript is generally well written, there are a few sentences key to understanding the results of the study that could be made clearer grammatically - I've highlighted a few below but the journal editor may be able to provide further assistance with respect to this.

Minor Comments - Figures

Generally, the formatting across figures could be more consistent i.e. font size, axis size etc.

Response: We have revised all figures and made all figures similar.

Figure 1 - Remove the question marks from around Age (Months).

Response: We revised it.

Figure 2 - This is a percentage not a proportion.

Response: We have revised the figures

Figure 3 - The figure legend does not accurately describe what the figures are. I think figure 3c is all of the samples combined and 3a and 3b are samples from hospitalised and non-hospitalised patients respectively but this should be made clearer.

Response: We have revised the figures. We have added the information for Figure 3a, 3b, and 3c.

Figure 3 - Is there any reason why 3b and 3c are stacked bar charts and 3a is not? The data is all of the same type and would it would be easier to compare between hospitalised and healthy strains if the data is presented in the same way.

Response: we have revised the figures to similar presentation.

Figure 3 - Figure 3a y-axis shouldn't go above 100%.

Response: we have revised the figure

Figure 3 - Figure legend should probably provide the full names of the antibiotics abbreviations used.

Response: we have added the description of antibiotics abbreviations used.

Figure 4 - I would advise including some axis-lines on these figures.

Response: We have revised the figures according to reviewer #1.

Figure 4 - Figure legend b) and c) say the same thing.

Response: We have revised the legend of figure 4b and 4c

Figure 4 - Y-axis could have either number of isolates or isolates (n).

Response: thank you for your suggestion, we will make the information similar as number of isolates (n)

Minor Comments - Manuscript

Line 27 - in children and adults.

Response: thank you for your suggestion, we have added the information.

Line 27-28 - the authors say "Streptococcus pneumoniae commonly causes pneumonia in children" and with the next sentence say there is a lack of reports for Streptococcus pneumoniae causing pneumonia in children. Do they mean there is a lack of information on whether particular serotypes are responsible?

Response: Correct, we have revised the sentence to include the serotypes.

Line 37 - should be 9.2% not 9,2%.

Response: we have revised the commas to decimals

Line 41 - most isolates.

Response: we have revised the manuscript accordingly.

Line 62 - as one of the.

Response: we have revised the manuscript as suggested.

Line 95 - the company producing nylon swabs should be included.

Response: we have added the company name.

Line 176 - the majority are not susceptible to oxacillin and co-trimoxazole.

Response: we have revised the manuscript according to your suggestion.

Line 177-179 - I think this would be clearer if stated that all isolates were resistant to oxacillin and co-trimoxazole rather than 'less susceptible'.

Response: we revised to resistant instead of less susceptible.

Line 177-179 - Not sure what "tetracycline (100%)" means here…around half of the isolates in this data appear to be susceptible to tetracycline?

Response: we have revised the percentage according to the figure, the percentage should be 67%, and it is already revised (line 184-187).

179 - 183 - I think this needs to be more carefully worded to make it clear that "less susceptible" means fewer strains that are susceptible to the antibiotic. "vancomycin is found susceptible for all isolates is also slightly confusing - something like "All isolates from healthy children and children with pneumonia are susceptible to vancomycin" might make this clearer.

Response: we have revised the less susceptible to resistant to be more obvious.

Line 188 - most common.

Response: we have revised it.

Line 204 - symptoms.

Response: We revised it.

Line 205 - Another explanation could be an age effect? Most children with pneumonia were under the age of 1 and most healthy children were older?

Response: It is also possible explanation because the age of 1-5 has higher prevalence of colonization.

Line 207 - *S. pneumoniae*should be italicised.

Response: We revised it.

Line 215 - malnourished.

Response: We revised it.

Line 214 - 216 - I would avoid using "this study" as readers might assume the results discussed in this sentence were produced by the authors and not in reference to another study.

Response: We revised it.

VERSION 1

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000584.v1.5

John Munnoch; University of Strathclyde, SIPBS, UNITED KINGDOM, Glasgow

Date report received: 03 April 2023
Recommendation: Minor Amendment

Comments: The work presented is clear and the arguments well formed. This study would be a valuable contribution to the existing literature. This is a study that would be of interest to the field and community. The reviewers have highlighted minor concerns with the work presented. Please ensure that you address their comments.

Reviewer 2 recommendation and comments

https://doi.org/10.1099/acmi.0.000584.v1.3

Anonymous.

Date report received: 22 March 2023
Recommendation: Minor Amendment

Comments: In this manuscript, the authors set out to address a lack of information on the carriage of Streptococcus pneumoniae, a causative agent of pneumonia, in healthy and hospitalized children in Indonesia. They also attempt to identify the serotypes circulating in these cohorts and test their susceptibility to antimicrobials. I have no major concerns about the manuscript - the methods are well articulated and the authors have done what they set out what they attempted to do, namely: describe the prevalence, serotype frequency, and antimicrobial susceptibilities of S.pneumoniae in cohorts of healthy and hospitalised children in the Padang region of Indonesia. They ultimately find that few hospitalised children actually carry S.pneumoniae in the naso-pharynx, identify the most common serotype of S.pneumoniae found in the these cohorts, and provide support for a recent study suggesting that S.pneumoniae is commonly resistant to a commonly used antimicrobial (co-trimoxazole). There are significant age differences between the hospitalized and healthy cohorts that could account for differences in S.pneumoniae prevalence but the authors acknowledge and discuss this in the manuscript. They also discuss their findings in relation to other studies reporting S.pneumoniae carriage and antimicrobial susceptibility in the wider region. I only have a few minor comments, most of which concern the formatting and presentation of the manuscripts figures. While the manuscript is generally well written, there are a few sentences key to understanding the results of the study that could be made clearer grammatically - I've highlighted a few below but the journal editor may be able to provide further assistance with respect to this. Minor Comments - Figures Generally, the formatting across figures could be more consistent i.e. font size, axis size etc. Figure 1 - Remove the question marks from around Age (Months). Figure 2 - This is a percentage not a proportion. Figure 3 - The figure legend does not accurately describe what the figures are. I think figure 3c is all of the samples combined and 3a and 3b are samples from hospitalised and non-hospitalised patients respectively but this should be made clearer. Figure 3 - Is there any reason why 3b and 3c are stacked bar charts and 3a is not? The data is all of the same type and would it would be easier to compare between hospitalised and healthy strains if the data is presented in the same way. Figure 3 - Figure 3a y-axis shouldn't go above 100%. Figure 3 - Figure legend should probably provide the full names of the antibiotics abbreviations used. Figure 4 - I would advise including some axis-lines on these figures. Figure 4 - Figure legend b) and c) say the same thing. Figure 4 - Y-axis could have either number of isolates or isolates (n). Minor Comments - Manuscript Line 27 - in children and adults. Line 27-28 - the authors say "Streptococcus pneumoniae commonly causes pneumonia in children" and with the next sentence say there is a lack of reports for Streptococcus pneumoniae causing pneumonia in children. Do they mean there is a lack of information on whether particular serotypes are responsible? Line 37 - should be 9.2% not 9,2%. Line 41 - most isolates. Line 62 - as one of the. Line 95 - the company producing nylon swabs should be included. Line 176 - the majority are not susceptible to oxacillin and co-trimoxazole. Line 177-179 - I think this would be clearer if stated that all isolates were resistant to oxacillin and co-trimoxazole rather than 'less susceptible'. Line 177-179 - Not sure what "tetracycline (100%)" means here…around half of the isolates in this data appear to be susceptible to tetracycline? 179 - 183 - I think this needs to be more carefully worded to make it clear that "less susceptible" means fewer strains that are susceptible to the antibiotic. "vancomycin is found susceptible for all isolates is also slightly confusing - something like "All isolates from healthy children and children with pneumonia are susceptible to vancomycin" might make this clearer. Line 188 - most common. Line 204 - symptoms. Line 205 - Another explanation could be an age effect? Most children with pneumonia were under the age of 1 and most healthy children were older? Line 207 - S.pneumoniae should be italicised. Line 215 - malnourished. Line 214 - 216 - I would avoid using "this study" as readers might assume the results discussed in this sentence were produced by the authors and not in reference to another study.

Please rate the manuscript for methodological rigour
Good

Please rate the quality of the presentation and structure of the manuscript
Satisfactory

To what extent are the conclusions supported by the data?
Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

Reviewer 1 recommendation and comments

https://doi.org/10.1099/acmi.0.000584.v1.4

Logan Mackie; University of Strathclyde Institute of Pharmacy and Biomedical Sciences, 161 Cathedral Street, Glasgow, UNITED KINGDOM

Date report received: 09 March 2023
Recommendation: Minor Amendment

Comments: 37 - Change comma to decimal point. 55 - 808,000 out of what, in what country? 60 - 12% of the 16%? Or 12% of all total deaths 60 - 9.5% of pneumonia? Or the 10.7%? 62-64 - References 95 -Cat number but no supplier ref 95- 96 - in how much media? Should be percentages. 101 - STGG abbreviation ? what is this? 102 - What is the enrichment media comprised of? what is Todd-Hewit broth comprised of? 104 - What is the blood agar comprised of? 108 - What is inoculated media comprised of? 113 - Decimal point instead of comma in percentages. 115 - Replace decimal point with comma in percentages. 116 - What is in the TE buffer? 120 - Make the rpm number the same as previous style. 130-133 - These should be concentrations rather than weights. 130 - Reference for the method. 156 - Decimal point instead of comma at 12.5%. 142 -150 - 131 patients mentioned in text versus 130 in table, extra patient is unaccounted for? Table 1- Numbers don't always add up to 65 or the full cohort number, but no explanation provided. Figure 1 - Y-axis should extend to 100% and axis line is missing. Y-axis label should be clearer e.g. % of children. No stats provided on the graphs, and blue bars e.g. hospitalised patients 13-24 months looks about double that of the other two age brackets -the results text mentions that there is a difference but no statistical analysis to back it up. Figure 1 legend - S. pneumoniae Figure 2a - Y-axis should extend to 100%, no line for Y-axis, Y-axis label should be clearer. Figure 2b - No line for Y-axis Figure 3a - No line for Y-axis, units on Y-axis should have decimal point rather than comma, Y-axis needs label, different style of graph compared to figure 3b, 3c. Figure 3b, Figure 3c - Y-axis needs label to clarify what percentages are. Figure 3 legend - Explain abbreviations or include abbreviations of antimicrobials in the results text, no explanation of what figure 3c shows. 180 -182- confusing wording, state that the strains are less susceptible to these compounds rather than the compounds have been less susceptible. 186 - Explain what MDR is an abbreviation of. Figure4a - Might be helpful to state which antibiotics in figure legend, Y-axis has no line, Y-axis needs label. X-axis label could be clearer e.g. number of antibiotics resistant per strain. 188 - Change commonest to most common Figure 4b, 4c - Key should be clearer e.g. state that this is number of antibiotics in the graph, Y-axis has no line. 208 - Change comma in 54,5% to decimal point, same with 62,5% 212 - Change comma in 13,9 to decimal point 223-224 - Clarify that this is a test to diagnose pneumonia and the 90.3% was correct identification 234 - Change to "is found to vary" rather than is found vary 235 + 236 + 244- Change commonest to most common. 252- Ampicillin and gentamycin is mentioned but previous history of antibiotic administration could not be provided. clarify that this is inferred based on common treatment routine. 309-310 - Reference 4 style is not consistent with other references 248-250 - Children under 1 year of age less likely to be in day-care? Which may explain some results e.g. older children more likely to be exposed to S.pneumoniae.

Please rate the manuscript for methodological rigour
Satisfactory

Please rate the quality of the presentation and structure of the manuscript
Satisfactory

To what extent are the conclusions supported by the data?
Partially support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

SciScore report

https://doi.org/10.1099/acmi.0.000584.v1.1

iThenticate report

https://doi.org/10.1099/acmi.0.000584.v1.2