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Bacterial Etiology of Respiratory Tract Infections among Ambulatory School Children in Moshi Municipality, Tanzania

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Abstract: Background: Respiratory tract infections are the leading cause of morbidity and mortality in children worldwide. Management of respiratory tract infections poses a challenge in developing countries particularly due to limited resources for bacterial identification. The objective of this study was to describe bacterial etiological agents causing respiratory tract infections and their resistance patterns among ambulatory school children in Moshi municipality, Tanzania. Methodology: A cross sectional community based study was conducted, from January to March 2014 in 4 primary schools in Moshi Municipality. All school children available at the time of study were assessed for respiratory symptoms, those with self-reported respiratory tract symptoms for four days or more were eligible for participation. While those on medication and those who completed oral medication within 15 days prior to screening were excluded. Nasopharyngeal and throat swabs were collected from eligible children. Isolates were tested for sensitivity against commonly used antibiotics. Results: Of 2,016 screened school children, 474 (23.5%) had respiratory tract symptoms. Respiratory tract bacterial pathogens were isolated from 123 (73.7%) of 167 children whose nasopharyngeal and throat swabs were collected, three children did no show for swab collection. *S. aureus* was the most prevalent isolate 68 (55.3%) followed by *S. pneumoniae* 43 (35.0%), and the least prevalent isolate was *K. pneumoniae* 7 (5.7%). Majority of isolated upper respiratory tract bacteria were resistant to ampicillin. *S. pneumoniae* exhibited the highest rate of the resistance whereby, 33 (91.7%) isolates were resistant to ampicillin. However, the resistance of isolates to cotrimoxazole was found to be low. Gentamicin and ceftriaxone were effective against most isolates. Conclusion: Prevalence of respiratory tract symptoms was high among ambulatory school children who were presumed to be healthy. The observed high resistance of isolates might be due to unnecessary prescription of antibiotics, and counterfeit drugs. There is a need to strengthen school health program, in order to identify children with respiratory tract infections and refer them to a health facility for further evaluation.

Keywords: Respiratory Tract Infections, Bacterial Pathogens, Resistant Pattern

1. Background

Respiratory tract infections (RTI) are infections that affect the air passages, including the nasal passages, the bronchi and the lungs [1]. RTIs are classified into upper respiratory tract infection and lower respiratory tract infection. Most upper respiratory tract infections are caused by virus (90.0%) followed by bacterial infections while co-infection occurs in 35-41% of cases [2,3]. Although viral RTIs are the commonest, bacterial RTIs are associated with severe morbidity, and high rates of mortality. The most frequent isolated bacterial pathogens causing respiratory tract infection in children are; *S. pneumoniae, S. aureus, K. pneumoniae, S. pyogenes, H. influenzae and M. pneumoniae* [2,3,4,5,6].

RTIs are the most frequently reported infections in all age groups with acute respiratory tract infection (ARTI) being the leading cause of morbidity and mortality worldwide in children [7]. Worldwide about 30 to 50% of paediatric medical consultations are due to RTIs, resulting into 20 to 40% of hospitalization and 50% of them die of pneumonia [8]. It is estimated that in 2010, 1.4 million children died from RTIs with 11.9 million episodes of severe and 3.0 million episodes of very severe ARTIs [9]. According to WHO, pneumonia alone kills 1.1 million children each year accounting for 18% of all deaths of children worldwide [1], and 50% of these deaths take place in Sub-Saharan Africa (SSA). Tanzania is among 15 countries with highest incidence of ARTIs in the world, with an estimated 1.9 million cases each year in under five [4].

RTIs can lead to a number of complications if left unattended, 12% children with ARTIs tend to develop complications [10]. Some of the known complications are; retropharyngeal abscess, empyema, bronchiectasis, meningitis, brain abscess, post-streptococcal glomerulonephritis and acute rheumatic fever [11,12].

Children with RTIs respond very well to antibiotics treatment but only 30% of children receive appropriate antibiotic [1]. There is wide spread miss use/over-use of antibiotics treatment especially for children in developing countries which may lead to just another challenge of drug resistance [13]. For instance, between September 2010 and March 2011, 80.0% of children with cough in Moshi were prescribed antibiotic inappropriately [14]. Furthermore, researchers from Tanzania found that; antibacterial were available in unauthorized drug outlets, and were dispensed without a prescription [15]. Reports from different countries, including those neighboring Tanzania, have reported resistance against recommended antibiotics for respiratory tract bacterial pathogens. For instance resistance to cotrimoxazole ranged from 9.1 to 83.5% [16,17] while that of penicillin at 3.3 to 93.3% [5,18].

In Tanzania and most developing countries, there is limited publication on the burden of RTIs among primary school children. Management of RTIs pose a challenge due to limited resources for the identification of the etiological agent and therefore administration of antibiotic is not guided by bacteriological sensitivity to the antibacterial agents, which increases risk of antibiotic resistance. This study aimed at providing evidence on the burden of RTIs among primary school children, identifying the common bacterial isolates and sensitivity pattern of isolated pathogens, thus to raise awareness and guide the empirical management of respiratory tract infections in children who are at school and presumed to be healthy.

2. Methods

2.1. Study Population

Moshi municipality has a total of 35 government primary schools, of them 4 were selected randomly for participation. At the time of screening, the total number of pupils in the selected four primary schools in Moshi municipality was 2,016. After written consent from respective school head teacher, all children from the selected school were screened for respiratory symptoms (cough, chest pain, chest tightness, wheezing, running nose, sneezing, and painful swallowing/sore throat). Study physician screed school children by inquiring the presence or absence of any respiratory symptom and the duration of the symptom (symptom for four days or more), and 268 children were eligible to participate in the study. Among them 170 (63.4%) children were randomly selected for bacteriological study for which nasopharyngeal and throat swabs were collected for laboratory analysis. In each class, children with respiratory symptoms were identified, if the number of those meeting inclusion criteria was 6 or less all were invited for participation while in classes were the number of eligible children was more than 6, randomly 6 were selected for participation. Among them, three pupils did not show up for swab collection; therefore, isolates were done from 167 pupils with self-reported respiratory symptoms. Children on antibiotic medication and those who completed antibiotic medication within 15 days prior to screening were excluded from the study.

2.2. Collection of Nasopharyngeal and Throat Swabs

Sterile polyester tipped applicator swab (Puritan medical products company llc Guilford, Maine USA) was carefully inserted into either of child nostril, and gently rubbed against the turbinate by rotating the swab. The swab was inserted into Stuart's transport medium bottle, then shaft of swab was broken off, and tube recapped. Children were then asked to open their mouth and a spatula was used to press the tongue downward to the floor of the mouth. Sterile polyester tipped applicator was used to swab both of the tonsillar arches and the posterior nasopharynx, then the shaft of swab was broken off and tube recapped. Specimens were transported at room temperature to the Kilimanjaro Christian Medical Centre (KCMC) clinical laboratory within 4 hours from the time of collection for processing.

2.3. Laboratory Procedure

Nasopharyngeal and throat specimens were processed at KCMC Clinical laboratory microbiology department according to Standard Operating Procedures (SOP). A swab of each specimen was taken from Stuart's transport medium and inoculated on Blood agar (BA), Chocolate (CA) and

MacConkey agar (MCA) plates. Nasopharyngeal and throat swab from each participant was plated differently. The plates were incubated under micro-aerobic conditions in 5-10% CO_2 atmosphere (with exception of MCA) at 35-37^{0}C for 24-48 hours. Bacteria identification was performed based on culture morphology, culture characteristics, and Gram stain of the growing colonies on the plates followed by biochemical tests for species identification. For gram positive organisms; catalase test, coagulase test and hemolysis were used for species identification. While for gram negative; Triple Sugar Iron Agar (TSI), Indole test, Citrate utilization, Urease test and Kovac's oxidase test were used for species identification

Drug Susceptibility Test (DST) was performed using the Kirby–Bauer disc diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2010). A standardized inoculum of a bacterial isolate was swabbed onto the surface of a Mueller- Hinton agar plate. Filter paper disks impregnated with a standard amount of antimicrobial agents was placed on the agar, and then incubated overnight. The diameter of the zone of inhibition was measured around each disk. The zone sizes were compared to tables in the disc-diffusion tests and MIC-determination from the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2010), to establish a qualitative report of susceptible, intermediate or resistant to each of the antimicrobial agents tested. The susceptibility of isolates were tested against the following drugs; ampicillin (10µg); chloramphenicol (30µg); gentamicin (10 µg); ceftriaxone (30µg); cotrimoxazole (1.25/23.75 µg).

2.4. Statistical Analysis

Data were double entered into EpiData 3.1 then exported to STATA/IC version 12.0 statistical software (StataCorp, U.S.A), for analysis. Chi square test and Fisher's exact test (when the expected frequency was less than 5) was used to investigate the association of the variables. All tests were two sided with P values $\leq$ 0.05 regarded as statistically significant.

3. Ethical Consideration

KCMC Research Ethical Committee approved the study and Permission from both Moshi Municipal Director and Moshi Municipal primary schools education officer to conduct the study was granted. The aim of this study was explained at each ward health committee where the selected schools are located and verbal approvals were granted from these committees. Since the study included screening to all children in specific classes similar to other school program, ethics committee waived the need for parental consent and approved obtaining verbal assent from the school children, and head of school signed informed consent for their schools to participate in this study.

4. Results

In total 2,016 pupils were screened, among them, 474 (23.5%) had respiratory tract symptoms. The female to male ratio were almost equal, female (51.8%). Majority presented with cough (80.0%), running nose (61.2%), chest pain (57.7%) and sneezing (56.5%). The mean age (±SD) of participants at enrollment was 10 (±2.1) years. Most of study participants were from school B (31.2%) followed by school D (26.5%). Regarding the class of study, more participants were from class five (16.5%). Table 1 below shows the general characteristics of study participants.

Table 1. *General characteristics of study participants (N=170).*

Variables	N	%
Sex		
Male	82	48.2
Female	88	51.8
Age categories, years		
6-9	66	38.8
10-12	80	47.1
13-15	24	14.1
Mean age (±SD) 10 (±2.1)		
School		
A	30	17.6
B	53	31.2
C	42	24.7
D	45	26.5
Education, class		
One	21	12.4
Two	23	13.5
Three	24	14.1
Four	23	13.5
Five	28	16.5
Six	25	14.7
Seven	26	15.3

Among children with respiratory tract infection, nasopharyngeal and throat swab were collected in 167 pupils who met inclusion criteria. Of the 167 pupils whose nasopharyngeal and throat swabs were tested for bacterial isolates and antibiotic sensitivity, 123 (73.7%) had one or more bacteria isolates with *S. aureus* 68 (55.3%) and *S. pneumoniae* 43 (35.0%) being the most prevalent upper respiratory tract isolates with *K. pneumoniae* 7 (5.7%) being the least isolate. Figure 1 below shows the prevalence of isolated upper respiratory tract bacterial pathogens.

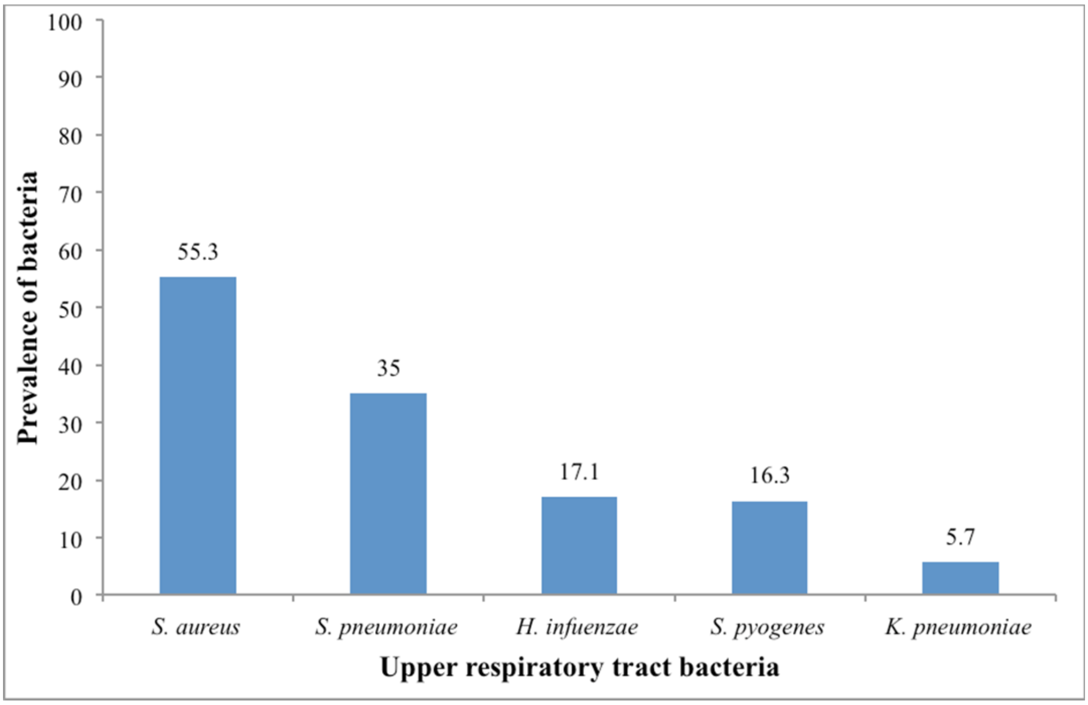

Figure 1. *Prevalence of upper respiratory tract bacterial isolates from school children with respiratory tract symptoms (n=123).*

Most of isolates were from children aged 10-12 years, in which 30 (44.1%) of *S. aureus* and 5 (71.43%) of *K. pneumoniae* were isolated from this age group. In general, there was statistical significant difference in the prevalence of *S. pyogenes* and *S. pneumoniae* between age groups and isolated bacterial pathogens (P<0.05) as shown in Table 2 below.

Table 2. *Age distribution of children with respiratory tract symptoms by bacterial pathogens (n=123).*

Isolate	Age categories in years n (%)			p-value*
	6-9	10-12	13-15	
S. pneumoniae				
Yes	15 (23.4)	15 (19.0)	13 (54.2)	0.012
No	49 (76.4)	64 (81.0)	11 (55.8)	
S. Aureus				
Yes	26 (40.6)	30 (38.0)	12 (50.0)	0.747
No	38 (59.4)	49 (62.0)	12 (50.0)	
K. pneumoniae				
Yes	1 (1.6)	5 (6.3)	1 (4.3)	0.060
No	65 (98.4)	74 (93.7)	23 (95.8)	
S. pyogenes				
Yes	11 (17.2)	9 (11.4)	0	0.028
No	53 (82.8)	70 (88.6)	24 (100)	
H. influenzae				
Yes	8 (12.5)	10 (14.5)	3 (12.5)	1.000
No	54 (87.5)	69 (85.5)	21 (87.5)	

*Chi squared p value for linear trend

Most of *S. pneumoniae* isolates were from pupils in school A, 18 (41.9%) and most of *S. pyogenes* were from pupils in school B, 11 (55.0%), while there was no *S. pyogenes* isolated from pupils in school A. Similarly, there was no *K. pneumoniae* isolated from pupils in school D. There was no statistically significant difference in the prevalence of respiratory tract bacterial isolates from these schools (Fisher's exact >0.05). See Table 3 below.

Table 3. *Distribution of respiratory tract bacterial isolates by schools (n=123).*

Schools n (%)					
Isolates	**A**	**B**	**C**	**D**	**Total**
S. pneumoniae	18 (41.9)	7 (16.3)	8 (18.6)	10 (23.2)	43 (100)
S. aureus	12 (17.7)	19 (27.9)	17 (25.0)	20 (29.4)	68 (100)
K. pneumoniae	3 (42.9)	1 (14.2)	3 (42.9)	0 (0.0)	7 (100)
S. pyogenes	0 (0.0)	11 (55.0)	5 (25.0)	4 (20.0)	20 (100)
H. influenzae	6 (28.6)	3 (14.3)	7 (33.3)	5 (23.8)	21 (100)
Total	39 (24.5)	41 (25.8)	40 (25.2)	39 (24.5)	159 (100)

Of the isolated 159 bacteria, 134 isolated were subjected to antimicrobial susceptibility testing. Among the gram positive bacteria, resistance to ampicillin was high; 33 (91.7%) for *S. pneumoniae* and 53 (84.1%) for *S. aureus*. The resistant of *S. pneumoniae* and *S. aureus* to cotrimoxazole was 25% and 9.5%, respectively. Gentamicin and ceftriaxone were the most effective antibiotics, whereby; almost all isolated *S. pyogenes* and *H. influenzae* were sensitive to gentamicin while all isolated *H. influenzae* were sensitive to ceftriaxone with only 1 (2.8%) of *S. pneumoniae* being resistant to ceftriaxone. See Table 4 below.

Table 4. *The antimicrobial resistance pattern of isolated bacteria from children with respiratory symptoms.*

Antimicrobial agent (concentration)	**Resistance pattern n (%)**				
	S. pneumoniae **n=36**	***S. Aureus*** **n=63**	***K. pneumoniae*** **n=7**	***S. pyogenes*** **n=17**	***H. influenzae*** **n=11**
Ampicillin (10 µg)	33 (91.7)	53 (84.1)	6 (85.7)	15 (88.2)	7 (63.6)
Chloramphenicol(30µg)	6 (16.7)	12 (19.0)	2 (28.6)	7 (41.1)	1 (9.0)
Gentamicin (10 µg)	4 (11.1)	6 (9.5)	1 (14.3)	0	0
Ceftriaxone (30 µg)	1 (2.8)	8 (12.7)	2 (28.6)	1 (5.9)	0
Cotrimoxazole (10 µg)	9 (25.0)	6 (9.5)	2 (28.6)	3 (17.7)	4 (36.4)

5. Discussion

There was high prevalence of respiratory tract infections in school children who otherwise were regarded as healthy; for every four children there was one with respiratory tract symptoms. The risk of transmitting the respiratory pathogens is very high considering the overcrowding of children in the class. Children with respiratory tract infection may transmit the pathogens to other children; with a possibility of a new infected child to take pathogens back home risking vulnerable groups such as under-five, and old people [19]. Parents and the community at large disregard respiratory tract symptoms and a good number of these infections will go without treatment, putting 12% of these children at a risk of developing complications [9,10]. At school where these children spend more time, there are no well stipulated health programs or screening program to identify children with respiratory tract infections. In fact the schools are good strategic points which can help in the fight against these common but potentially serious infections, as far as complications are concerned.

Though there are sporadic studies in this age group, our results are similar with those from north-east Tanzania among 1-18 years children, where the prevalence of respiratory tract symptoms was 22% [20,21]. Similarly, a study from Kenya reported high prevalence of respiratory tract infections in school children [22]. However, a study from India reported a low prevalence of respiratory tract infections among school children [23]. The prevalence variation might have been attributed to the sampling frame, age category of study participants and seasonal variation during the different study period.

S. aureus was identified as the most frequent bacterial isolate from children with respiratory tract symptoms. Many studies in the past have identify *H. influenzae* and *S. pneumoniae* as the most prevalent respiratory tract bacterial pathogens causing respiratory tract infections, contrary to this study [3,5]. The shift in the bacterial etiological agent might have been due to the inclusion of school-aged children; whereas, most of the previous studies were from under-five children. Secondly, the introduction of pneumococcal vaccine and *H. influenzae* type b vaccine in the national program of immunization might have contributed to the low prevalence of these respiratory tract bacterial pathogens compared to *S. aureus*. With the evidence from Gambian study, where introduction of Hib vaccine was associated with a decline in Hib carriage among young children from 12% to 0.25% [24]. This might have played role in low transmission rate of *H. influenzae* and hence, low prevalence in school aged children. Similarly, studies from Italy, Netherlands and Lithuania have reported a high prevalence of *S. aureus* ranging from 35.0% to 67.3% [25–28]. However a study from Nigeria reported a low prevalence (5.4%) of *S. aureus* among children with respiratory tract symptoms [5]. It does appear that the prevalence of *S. aureus* in healthy children and children with symptoms varies widely by geography, age and season.

The second most common isolate was *S. pneumoniae* (35.0%); several authors have reported similar findings from different parts of the world. In most of these studies *S. pneumoniae* was identified as leading cause of respiratory tract infections in children less than 5 year with a shift in school children. A study from Kenya, reported a similar prevalence 35.7% of *S. pneumoniae* [29]. Lower prevalence have been reported with researcher from Turkey and Nigeria, they found the prevalence of 23.4% and 6.8%, respectively [17]. However, our prevalence of *S. pneumoniae* does appear to be lower compared with those from India, Gambia and Australia which reported prevalence at 53.4%, 85.1% and 67.0%, respectively [18,20,25].

The study has shown a high prevalence of *S. pyogenes* in this age group, with many children presenting with sore throat. When streptococcal pharyngitis infections are left unattended within 9 days from the onset of symptoms, the chances of developing complications or healing with sequel is very high. Some strains of Group A streptococcal are rheumatogenic causing acute rheumatic fever and 60.0% of acute renal injury (ARF) cases end up with rheumatic heart disease. Given the fact that the average duration of symptoms was above 9 days and it is estimated that 3-5% of population has an inherent susceptibility to ARF. A number of children in Tanzania suffering from sore throat are at a risk of developing ARF and some of them will complicate into rheumatic heart disease (RHD). The risk of developing ARF increases with recurrent sore throat infections. Our projecting is supported with 2008-2009 cardiac surgery experience at Muhimbili National Hospital; data have shown that 47.0% of performed cardiac surgeries were due to RHD [30]. Reports from developed countries have shown a decline in the incidence of RHD which might not be the case in developing countries, given the overcrowding in home and in classes, living conditions, poverty and poor accessibility to health care in these countries. With our findings, a number of children are at a risk of developing renal conditions later in their lives, since the risk of developing renal conditions in later life is three times higher with childhood post streptococcal glomerulonephritis and the risk increases with repeated episodes [31]. Studies from Nepal and Nigeria, have reported a similar high prevalence *S. pyogenes* in this age group [5,32].

In general most respiratory tract bacterial isolates were sensitive to ceftriaxone and gentamicin, with a very high resistance to ampicillin. The resistance to recommended and commonly used antibiotics for treatment of respiratory tract infections was high. This might be due to the fact that these drugs are readily available from drug outlets, cheap and easily accessible even without a prescription; with expected misuse. In most cases respiratory tract infections are treated empirically which just adds another risk for resistance development [15]. The risk of transmitting these resistant strains of respiratory tract bacteria is very high in the community since the community disregards respiratory tract infections. There is a lot of unnecessary prescription of antibiotics especially for acute viral respiratory tract infection, and the issues of counterfeit drugs, long shelf life and expired antibiotics are adding to this problem of resistance. Study results conform to studies from Uganda, Italy, Turkey and Nigeria which reported high resistance *S. pneumoniae, S. aureus, K. pneumoniae and S. pyogenes* against penicillin and its derivatives [5,16,17,25]. However, few studies from different countries have shown different results. Finding from Central Africa Republic, India and Gambia reported the resistance of *S. pneumoniae* against penicillin and its derivatives at 8.8%, 3.3% and 14.3% (18,24,33]. The seen variation in resistance of respiratory tract isolates against penicillin/ampicillin may be due to the geographical distribution of resistant strains and country specific policy on the accessibility and use of recommended antibiotics for respiratory tract infections and other bacterial infections.

6. Conclusion

In this study we found a high prevalence of respiratory symptoms among ambulatory school children who were presumed to be healthy, which raises the need for addressing the situation. This may be due to the overcrowding of children in the class, and the fact that these symptoms are disregarded by the parents. The observed high resistance of isolates might be due to increase in empirically treatment of respiratory tract infections with unnecessary prescription of antibiotics, and counterfeit drugs. There is a need to strengthen the available school health program, to be able to identify pupils with respiratory tract infections, and refer them to a health facility for further evaluations. The use of cotrimoxazole in the treatment of uncomplicated respiratory tract infections should be considered.

7. Strength and Limitations

One of the limitations of our study was the use of conversional culture method which has low sensitivity in detecting bacterial pathogen (swabs from 44 children with respiratory symptoms had no bacteria growth) and that the media used were not able to culture some of the known bacterial pathogens at this age group. But still we were able to demonstrate the burden of respiratory tract infections in the community where children are presumed to be healthy. This is one of the few community studies in Moshi municipality done among school children and the study has involved both clinical and bacteriology assessment which add to its strength.

Authors' contributions

JSN conceived the study, design, coordinated data collection, and led analysis, interpretation and manuscript preparation. CA participated in data collection, analysis and manuscript preparation. MS coordinated sample processing and participated in manuscript preparation.

BTM participated in interpretation of the findings and manuscript writing.

GSK participated in study design, interpretation of the findings, manuscript preparation and final review of manuscript.

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