ORIGINAL ARTICLE

A comparative profile of oropharyngeal colonization of *Streptococcus pneumoniae* and *Hemophilus influenzae* among HealthCare Workers (HCW) in a tertiary care hospital and non-healthcare individuals

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Keywords

Oropharyngeal carriage • *Streptococcus pneumoniae* • *Haemophilus influenzae* • *Health care workers*

Summary

Introduction. *Streptococcus pneumoniae and Hemophilus influenzae are two major bacterial human pathogens responsible for causing both acute respiratory tract and life threatening invasive infections. Oropharyngeal carriage of these isolates can lead to its transmission frequently in healthcare settings between patients and HealthCare workers (HCW) and also common among population living in crowded communities resulting in serious invasive infections. Furthermore, awareness about preventive measures including appropriate vaccination against these bacterial infections, oropharyngeal carrier status, prevalent serotypes and the antimicrobial susceptibility pattern these bacterial strains among HCW and Non-HCW in the community in India remains inadequate. Therefore the current study is aimed to understand the prevalence of oropharyngeal carrier status, prevalent serotypes and antimicrobial susceptibility profile of these organisms among HCW and non-HCW.*

Methods. *A total of 200 oropharyngeal swabs were collected from HealthCare Workers and 200 from Non-Health care individuals of age 18 to 70. Antimicrobial susceptibility profile was studied for Pneumococci and H. influenzae. Specific serotypes for the carrier isolates of Pneumococci were identified using primers appropriate to the prevalent serotypes by multiplex PCR.*

Results. *About 30% of the HCW were colonized with S.pneumoniae and H. influenzae ($P \leq 0.0001$). Out of which 19% of them were S.pneumoniae and 11% were H. influenzae. A total of 23% of the Non-HCW was colonized with S. pneumoniae and H. influenzae. Out of which 16% had pneumococcal carriage and 7% had H. influenzae. Individuals in the age group 56-70 years had significantly a greater prevalence rate when compared to young people ($P = 0.0014$). Thus in this study 30% of the HCW and 23% of the Non-HCW were colonized with S. pneumoniae and H. influenzae. Both Pneumococci and H. influenzae showed 100% susceptibility to Penicillin and other cephalosporins. However, Pneumococcal isolates from HCW showed better susceptibility towards erythromycin & clindamycin whereas isolates from Non- HCW showed better susceptibility towards ofloxacin and tetracycline. Serotypes detected in our study include 19F, 3, 1 and 5.*

Conclusions. *The present study gives a greater prevalence rate of S.pneumoniae and H. influenzae among HCW when compared to Non-HCW. This will definitely increase horizontal spread of infections and further accelerate the occupational risk. Increased carrier state prevalence among old age group underscores the importance of vaccination among these individuals.*

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) and *Haemophilus influenzae* (*H. influenzae*) remain as the most important bacterial respiratory tract pathogens [1, 2]. Humans are the only known asymptomatic carriers/reservoir [3]. The clinical infection of these bacteria is preceded by asymptomatic colonization of the human pharynx [4]. The pharyngeal mucosa is considered as the ecological niche for *S. pneumoniae* and *H. influenzae* which can extend their entry into the respiratory tract and further lead to invasive infections, like pneumonia a major cause of death, particularly in the elderly population [5, 6].

In the current study we have selected *S. pneumoniae* and *H. influenzae* because they are members of the normal microflora as well as being etiologic agents of common respiratory tract and disseminated infections [7]. Transmission occurs through direct contact with respiratory droplets from asymptomatic pharyngeal carrier, patients, or indirectly through fomites [8, 9]. Transmission occurs frequently in healthcare settings between patients and HealthCare workers (HCW) and also common among crowded communities through droplets [10, 11]. Other risk factors for the invasive disease caused by these bacteria include extremes of age, immunocompromised states and viral infections of the respiratory tract [12]. Awareness about preventive measures including appropriate vaccination remains inadequate [10]. Limited data exists with respect to prevalence of oropharyngeal carrier status, prevalent

serotypes and resistant patterns in HealthCare Workers and Non-HealthCare individuals in the community in India.

Materials and methods

Study design

Prospective observational study. Place of study: Department of Respiratory and Thoracic medicine and Department of Microbiology, Chettinad Hospital and Research Institute, Kelambakkam. Duration of the study: This was carried out in during a period of 6 months, May to Oct 2018. Sample size and study population: sample size was calculated using this formula:

$$\text{Sample size (n)} = \frac{Z^2\ P\ Q}{d^2}$$

A total of 200 oropharyngeal swabs were collected from HealthCare Workers and 200 from Non-Health care individuals of age 18 to 70. Data such as demographic details, antibiotic use, smoking habits, immunization and other health related details were collected through individual questionnaires. Healthcare Workers include doctors, nurses, housekeeping staff and laboratory workers. Non-Healthcare Workers (not exposed to healthcare settings) include white collar workers, fishermen, farmers, construction workers, driver, sweeper, tailor and others (home makers and other subjects currently not pursuing any job).

Inclusion criteria: Age between 18-70 years, both male and female, Health care workers from a tertiary care hospital, non-health care individuals (not exposed to healthcare settings) of age 18 to 70. *Exclusion criteria:* recent respiratory infections within 8 weeks, recent exposure or contact with individuals having respiratory infections, preexisting respiratory diseases, antibiotic usage for more than 2 weeks before taking oropharyngeal swab and immunocompromised individuals. This study was initiated after obtaining the approval from Institutional Human Ethical committee (IHEC) (Ref: no: 110/IHEC/03-18).

Sample collection

All participants were informed in detail about the study and written consent was obtained before collecting the samples from individuals who participated in the study. All participants were subjected to a detailed questionnaire and clinical examination. Oropharyngeal swabs were collected using sterile cotton swabs and the swabs were transported in Amie's transport medium to microbiology department within 4 hours. Swabs were streaked onto trypticase soy agar supplemented with 5% sheep blood agar containing 5µg of gentamicin and chocolate agar. Plates were incubated at 37°C aerobically in the presence of 5-10% CO_2 enriched air.

Bacterial identification

Pneumococci growing as alpha hemolytic colonies on blood agar were identified according to standard microbiological procedures like Gram's stain, optochin susceptibility test, bile solubility test and *H. influenzae* colonies growing as translucent colonies on chocolate agar were identified by phenomenon of Satellitism.

Antimicrobial susceptibility testing

Antimicrobial susceptibility profile was studied for *Pneumococci* and *H. influenzae* by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standards Institute. The antibiotics tested against the isolates include: penicillin, vancomycin, erythromycin, azithromycin, tetracycline, ofloxacin and cotrimoxazole for *S. pneumoniae*; ampicillin, cefotaxime, cefipime, meropenem and imipenem for *H. influenzae*.

Serotyping PCR for *Pneumococci*

Specific serotypes were identified using primers appropriate to the prevalent serotypes by multiplex PCR. Briefly multiplex PCR was set up in a 25 µl reaction mix. The thermal cycling condition is as follows: 95°C for 15 min (1 cycle), 94°C for 30 sec, 54°C for 90 sec, 72°C for 60 sec (35 cycles) and 72°C for 10 min (1 cycle). The primer sets included are given in (Tab. I).

Statistical analysis

The results were analyzed using IBM SPSS (version 21.0) software. Pearson's Chi Square test was done to analyze the association between the two groups (Healthcare and Non-Healthcare workers).

Results

A total of 400 oropharyngeal swabs 200 from each category i.e. Health care workers and Non-Healthcare workers were collected.

About 66% {132/200} of the HCW were in the age group of 18-35, 32% {64/200} of them in the age group of 36-55 and 2% {4/200} of them in the age group 56-70 (Fig. 1). Of the 200 HCW, 72% {144/200} were female and 28% {56/200} were male volunteers (Fig. 2). With respect to occupation wise distribution 51.5% {103/200} were doctors and nurses, 39.5% {79/200} were housekeeping staff and 9% {18/200} were laboratory workers (Fig. 3).

About 30% {60/200} of the HCW were colonized with *S.pneumoniae* and *H. influenzae* ($P \leq 0.0001$). Out of which, 19% {38/200}of HCW were *S.pneumoniae* and 11% {22/200} were *H. influenzae* (Fig. 4). About 10% {13.2/132} of HCW in the age group 18-35 and 9% {5.76/64} in the age group 36-55 had *pneumococcal* carriage whereas 3.5% {4.62/132} of HCW in 18-35 age group and 7.5% {4.8/64} of HCW in the age group 36-55 carried *H. influenzae* in their pharynx (Fig. 5). About 14% {20.16/144} of the females and 5% {2.8/56}of the males harbored *S. pneumoniae* in their oropharynx whereas 6.5% {9.36/144} of the females and 4.5% {2.52/56} of the males harbored *H. influenzae* (Fig. 6). Carrier state was found to be significantly higher in female volunteers when compared to males ($p = 0.0298$). Distribution of *S.pneumoniae* colonization among HCW

Tab. I. Set of primers included in the study.

Primers	Gen. bank accession n.	Primer sequence (5'-3')	Gene	Nucleotide position	Product size bp
1-f	CR931632	CTC TAT AGA ATG GAG TAT ATA AAC TAT GGT TA	*wzy*	9,935	280
1-r		CCA AAG AAA ATA CTA ACA TTA TCA CAA TAT TGG C		10,181	
3-f	CR931634	ATG GTG TGA TTT CTC CTA GAT TGG AAA GTA G	*gal U*	*9,020*	*371*
3-r		CTT CTC CAA TTG CTT ACC AAG TGC AAT AAC G		*9,360*	
5-f	CR931637	ATA CCT ACA CAA CTT CTG ATT ATG CCT TTG TG	*wzy*	6,123	362
5-r		GCT CGA TAA ACA TAA TCA ATA TTT GAA AAA GTA TG		6,450	
19F-f	CR931678	GTT AAG ATT GCT GAT CGA TTA ATT GAT ATC C	*wzy*	11,135	304
19F-r		GTA ATA TGT CTT TAG GGC GTT TAT GGC GAT AG		11,407	
8-f	CR931644	GAA GAA ACG AAA CTG TCA GAG CAT TTA CAT	*wzy*	11,193	201
8-r		CTA TAG ATA CTA GTA GAG CTG TTC TAG TCT		11,364	
14-f	CR931662	GAA ATG TTA CTT GGC GCA GGT GTC AGA ATT	*wzy*	7,959	189
14-r		GCC AAT ACT TCT TAG TCT CTC AGA TGA AT		8,119	

Fig. 1. Age wise distribution among HCW.

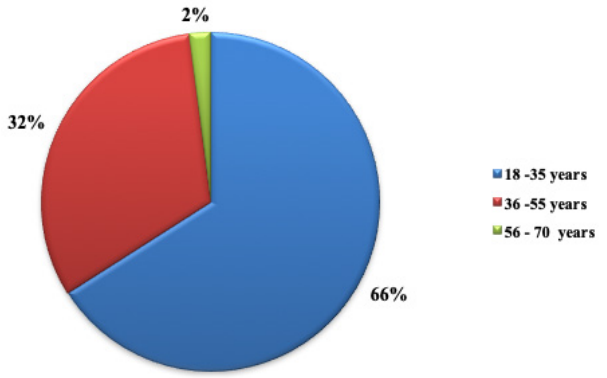


Fig. 2. Sex wise distribution of HCW.

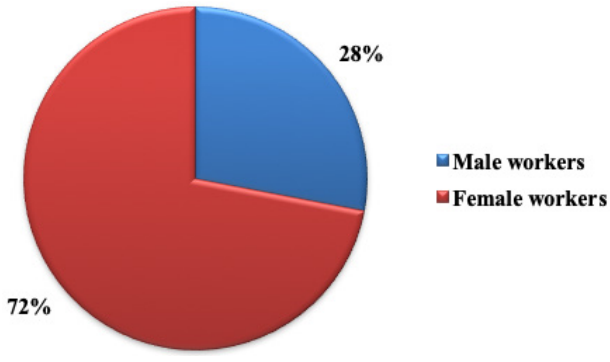


Fig. 3. Occupation status wise distribution among HCW.

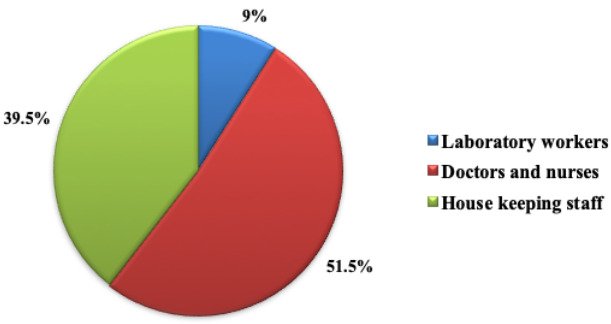


Fig. 4. Oropharyngeal colonization of *S. pneumoniae* and *H. influenzae* in HCW.

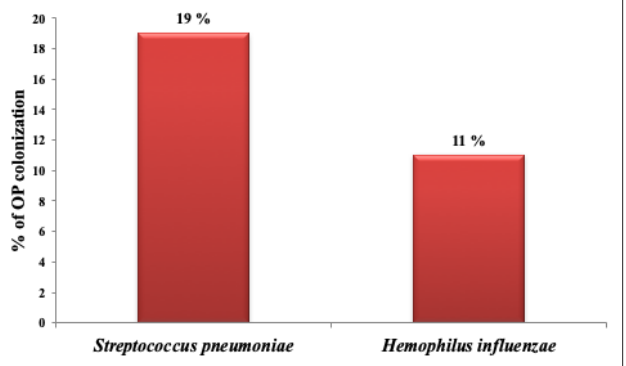
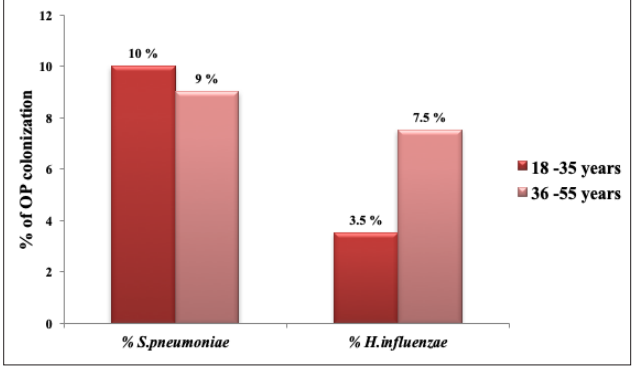


Fig. 5. Oropharyngeal colonization of *S. pneumoniae* and *H. influenzae* among different age groups of HCW.



belonging to different category include, 10% {10.3/103} of doctors and nurses, 8% {6.32/79} of housekeeping staff and 1% {0.18/18} of laboratory workers. Whereas distribution of *H. influenzae* include, 4.5%{4.635/103}, 6.5% {5.135/79} and 0% respectively (Fig. 7).

Among the Non-HCW 28% {56/200} of them belonged to the age group 18-35, 14% {28/200} were in the age group 36-55, 58% {116/200} in the age group 56-70 (Fig. 8). Out of the 200 Non-HCW, 59% {118/200} of the healthy volunteers were female and 41% {82/200} were male (Fig. 9). Among the non-HCW 42% {84/200} of them were white collar workers, 30% {60/200}

Fig. 6. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among male and female HCW.

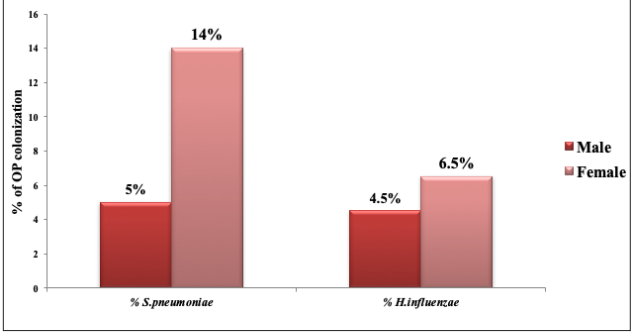


Fig. 7. % of Oropharyngeal colonization of *S. pneumoniae* and *H. influenzae* isolates among various category of HCW.

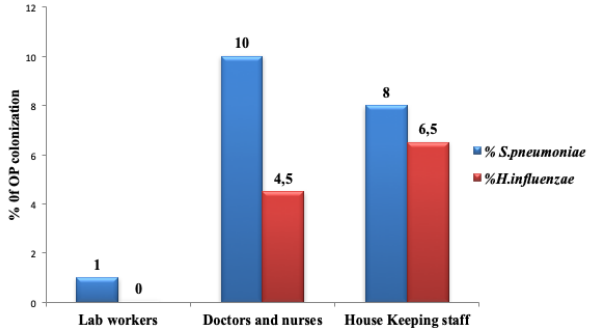


Fig. 8. Age wise distribution among Non-HCW.

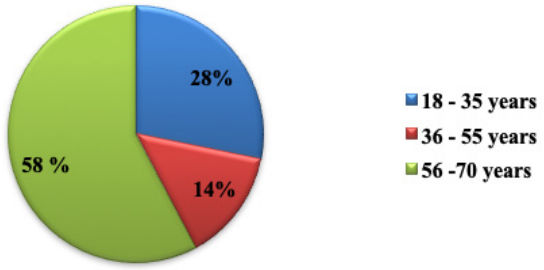
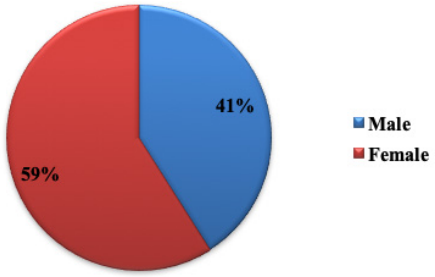
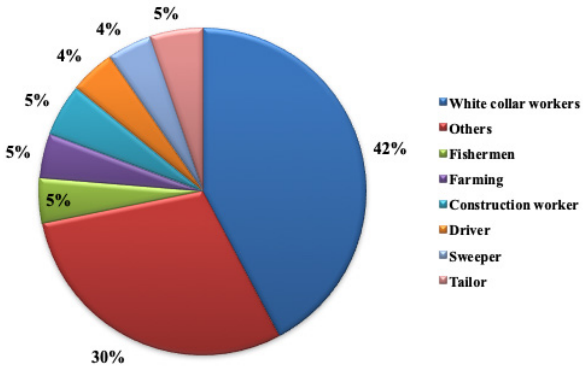


Fig. 9. Sex wise distribution of Non-HCW.



include home makers and other subjects not pursuing any job, 4% {8/200} fishermen, 4% {8/200} farmers, 5% {10/200} construction workers, 4%{8/200} drivers, 4% {8/200} sweepers and 5% {10/200} tailors (Fig. 10). A total of 23%{46/200} of Non-HCW were colonized with *S. pneumoniae* and *H. influenzae*. Out of which

Fig. 10. Occupation status wise distribution among Non-HCW.



16% {32/200} had pneumococcal carriage and 7% {14/200} had *H. influenzae* (Fig. 11).

In this study 3% {1.68/56} of Non-HCW in the age group 18-35, 4% {1.12/28} of Non-HCW in the age group 36-55 and 9% {10.44/116} of Non-HCW in the age group 56-70 harbored *Pneumococci*. Whereas 0.5% {0.28/56} of Non-HCW in age group 18-35, 2.0% {0.56/28} in the age group 36-55 and 4.5% {5.22/116} in the age group 56-70 had *H. influenzae* carriage (Fig. 12).

Individuals in the age group 56-70 years had significantly a greater prevalence rate when compared to young people

Fig. 11. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* in Non-HCW.

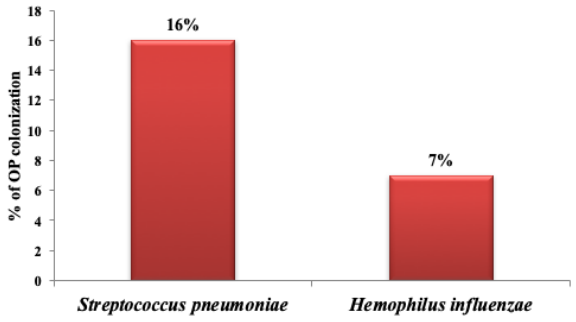
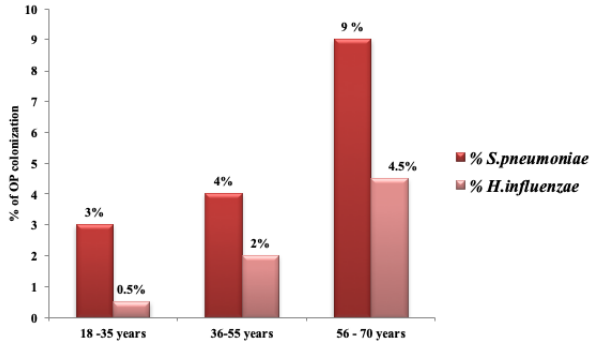


Fig. 12. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among different age groups of Non-HCW.



(P = 0.0014). About 9% {7.38/82} of the male and 7% {8.26/118} of the female Non-HCW was colonized with *Pneumococci* whereas 4.5%{3.69/82} of the male and 2.5% {2.95/118} of the females was colonized with *H. influenzae* (Fig. 13).

Colonization of *Pneumococci* among Non-HCW belonging to different occupation include white collar workers 5% {4.2/84}, others (home makers and other subjects not pursuing any job) 9% {5.4/60} , fishermen 2%{0.16/8} and sweepers nil. Non-HCW colonized with *H. influenzae* includes 1.5% {1.26/84}, 4.5% {2.7/60}, 0% and 1% {0.08/8} respectively (Fig. 14).

Thus in this study 30% {60/200} of the HCW and 23% {46/200} of the Non-HCW were colonized with *S. pneumoniae* and *H. influenzae* (Fig. 15).

Antimicrobial susceptibility pattern of *Pneumococci* and *H. influenzae* obtained from HCW & Non-HCW. The *Pneumococcal* isolates obtained from the healthcare workers showed 100% susceptibility to penicillin, 82% {31.16/38} to erythromycin, 73% {27.74/38} to clindamycin, 64% {24.32/38} to ofloxacin, 45% {17.1/38} to tetracycline and 100 % to vancomycin. Whereas the susceptibility pattern of *Pneumococcal* isolates from Non-HCW include 100% to penicillin, 47% {15.04/32} to erythromycin, 60% {19.2/32} to clindamycin, 87% {27.84/32} to ofloxacin, 67% {21.44/32} to tetracycline and 100 % to vancomycin (Fig. 16).

H. influenzae isolates obtained from Healthcare and Non-Healthcare workers showed 10% susceptibility to ampicillin, 85% to cefotaxime and 100% susceptibility to imipenem & meropenem (Fig. 17).

Representative *Pneumococcal* isolates obtained from Health care and Non-Healthcare workers were subjected to serotyping multiplex PCR. The serotypes obtained include 19F, 3, 1 & 5 (Fig. 18).

Fig. 13. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among male and female Non-HCW.

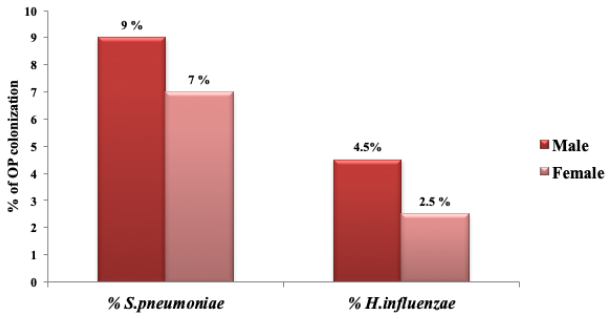


Fig. 14. Oropharyngeal colonization of *H. influenzae* and *S. pneumoniae* among Non-HCW belonging to different occupation.

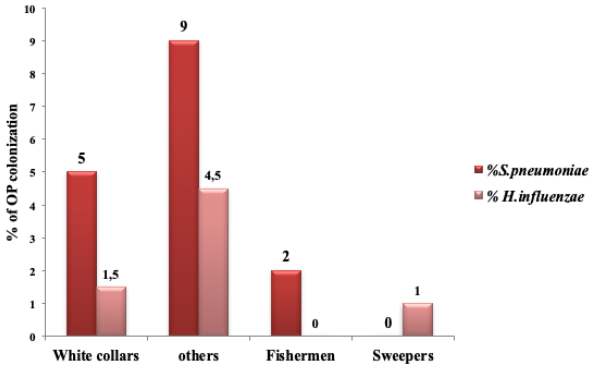


Fig. 15. *H. influenzae* and *S. pneumoniae* oropharyngeal carriage among HCW and Non-HCW.

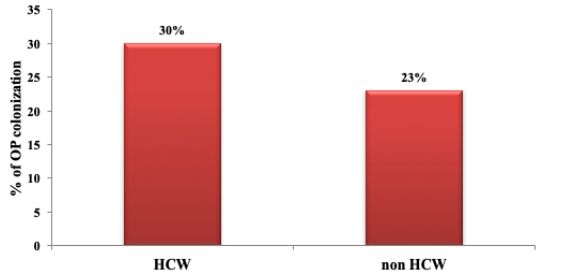


Fig. 16. Antibiotic susceptibility pattern of *Pneumococci* obtained from HCW and Non-HCW.

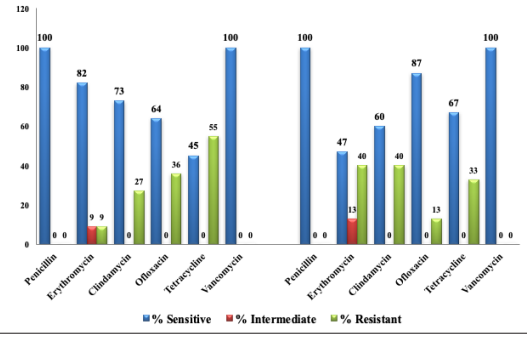


Fig. 17. Antibiotic susceptibility pattern of *H. influenzae* obtained from HCW and Non-HCW.

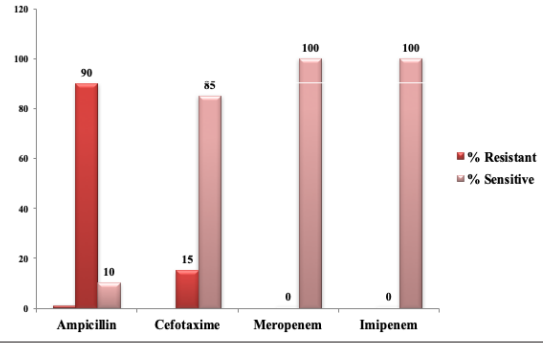


Fig. 18. Gel pictures representing different serotypes of *S. pneumoniae*.

Discussion

The upper respiratory tract is the ecological niche for many bacterial species. Colonization of the pathogens like *S. pneumoniae* and *H. influenzae* usually result in the horizontal dissemination of these pathogens to individuals within the community. The rates of bacterial colonization mostly depend on the age, occupation, geographical area and socioeconomic conditions [13-16]. Current study shows that the people belonging to 56-70 years had more colonization of *S.pneumoniae* and *H. influenzae* when compared to people belonging to age groups between 18-55 years and this was concordant with few studies [17]. This could be due to decreasing immunity with increasing age and associated chronic conditions.

Our study shows that oropharyngeal colonization was found to be more predominant among female health care workers when compared to male health care workers. This may be due to the predominant female population among health care workers especially with nursing and housekeeping staff. In the present study the *S. pneumoniae* and *H. influenzae* carrier state was found to be higher in healthcare workers (30%) when compared to Non-Health care workers (23%). This was similar to the study report by Hosuru Subramaniya 2016 in which about 65% of HCW and 32% of Non-HCW harbored these pathogens.

Among the health care workers, doctors, nurses and housekeeping staff had higher carrier rate when compared to laboratory workers. This was consistent with other reports [10]. This clearly depicts that higher rate of carriage among healthcare workers especially among doctors, nurses and housekeeping personnel may be attributed due to frequent exposure to hospital environment.

In our study, *Pneumococcal* isolates from HCW showed 100% susceptibility to penicillin and vancomycin whereas resistance was noted against erythromycin, clindamycin, ofloxacin and tetracycline as 9%, 27%, 36% and 55% respectively. However, isolates from non-HCW showed 100% susceptibility to both penicillin and vancomycin but 40% of the isolates showed resistance towards erythromycin & clindamycin, 13% towards ofloxacin and 33% were resistant to tetracycline. In our study, isolates from HCW showed better susceptibility towards erythromycin & clindamycin whereas isolates from non-HCW showed better susceptibility towards ofloxacin and tetracycline. A report by Goyal gives 100% sensitivity to quinolones, macrolides and 61% resistance to tetracycline [18]. Whereas another report states that 90-100% of the isolates were found to be susceptible to penicillin and erythromycin which was found to be consistent with our report [10]. *H. influenzae* isolates from HCW and non-HCW showed 90% resistance towards penicillin whereas only 15% of the isolates were resistant towards cefotaxime. All the isolates were susceptible to imipenem and meropenem. A study by Yunusa Thairu states that majority of the *H. influenzae* isolates were resistant to ampicillin whereas all the isolates were sensitive to third generation cephalosporins like ceftriaxone [19]. Serotyping done for representative isolates revealed the following serotypes, which include 1, 5, 3 and 19F. Another study done by Molander gives a report that the most common serotypes obtained from invasive infections include 1,5, 19F, 6B, 14 and 3 [20]. However Felipe Piedade Gonçalves Neves reports that the prevalent serotype among carrier isolates include 6B, 19F, 6A, 14, 15C and 23F [21]. A study from India states that serotype 1 and 3 are the prevalent serotypes in all age groups [22]. Another report from India gives serotypes 1, 3, 5, 19F, 8, 14, 23F, 4, 19A and 6B as the predominant serotypes. Since the oropharyngeal colonizers are responsible for the spread of invasive infection a thorough knowledge about the prevalent colonizing serotypes can further aid in improving the efficacy of currently available pneumococcal vaccine.

Conclusions

The present study gives a greater prevalence rate of *S. pneumoniae* and *H. influenzae* among HCW when compared to Non-healthcare workers. This will definitely increase horizontal spread of infections and further accelerate the occupational risk. Thus carrier status is a concern for both HCW and non-HCW which can pave way for increased morbidity and mortality rate. Increased carrier state prevalence among old age group underscores the importance of vaccination among these individuals. Serotyping of the carrier strains definitely will give a greater insight for the inclusion of the serotype as a vaccine candidate and also help in identifying its clinical significance.

Acknowledgements

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

APSR, MN, AGN and PS designed the study. AGN, APSR and PJ collected, processed and identified the strains. AGN, APSR, MN and PS participated in the manuscript revision. All authors of the manuscript gave their complete approval.

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