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Prevalence and Antibiotic Resistance of *Streptococcus pneumoniae* among Different Hospitals in Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between all authors. Author AKJ planned, designed, approved study concept and supervised in collection, assembly, possession of raw data, funds arrangement and valuable suggestions in article preparation. Author AJ performed data analysis, critical discussion and manuscript preparation. Author AMM carried out sampling, administrative, technical and material support to accomplish this study. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Present Study was directed to examine the pervasiveness, antibiotics resistance and susceptibility array in *Streptococcus pneumoniae* gram positive and gram negative isolated strains.
Methodology: In current study, 6549 suspected samples were proceeded to confirm pneumonia positive cases obtained from various hospitals. Gram positive and gram negative strains were isolated and characterized by conventional biochemical techniques. Antibiotic susceptibility test was carried out using the disk diffusion test according to the Clinical and Standard Laboratory Institute guidelines (CLSI). Further, streptococcus based isolates were investigated using MacConkey agar.

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Results: A total number of 6549 suspected clinically samples were collected and 3716 (56.7%) pneumonia cases were confirmed to be positive. More than half of the confirmed cases fall in the age of 20-65 years and male proportion was found to be 64%. Interestingly, 98.2% of the cases were hospital acquired pneumoniae (HAP). *P. aeruginosa* most widely recognized isolate both hospital and community acquired (CA) etiological agent contributed 18.4% and 32.2% proportion respectively.

Conclusion: Clinicians ought to know that average observational pneumonia treatment administrations may not function admirably because of the diverse microbes. This requires sputum examining before beginning treatment for recognizable proof and affectability testing subsequent to a solitary measurement of antimicrobial treatment preceding pneumonia finding might impede the discovery of the causative specialists.

Keywords: Streptococcus pneumoniae; prevalence; antibiotic; resistance; Saudi Arabia.

1. INTRODUCTION

Streptococcus pneumoniae is a key respiratory pathogen that is actively involved in couple of respiratory sickness including otitis media, pneumonia and sinusitis [1]. The prevalence of pneumococcal nasal pharyngeal asymptomatic colonization in the adults has been increased [2]. The biological corner for *S. pneumoniae* is the human nasopharynx. Transfer of pneumococci in the nasopharynx is more common in adults than young ones. The span of carriage is affected by host and pathogen-related components. The growing recurrence of hostile to microbial safe *S. pneumoniae* strains raises another risk to the treatment of disease [3]. In the mid of recent decade, antimicrobial resistance has expanded around the world [4]. Specifically, imperviousness to penicillin and the macrolides has spread rapidly among withdraws of *S. pneumoniae* [5].

Few studies have proposed a connection between pneumococcal macrolide resistance and treatment disappointment in patients with group cause respiratory tract infections (RTI) [6]. The development of Multi Drug Resistance safe *S. pneumoniae* (MDRSP) has been seen in different nations over the past decades. The developing resistance of *S. pneumoniae* to usually utilized antimicrobials underlines the critical requirement for immunizations to be utilized to control pneumococcal disease.

Current study focuses to determine any possible measurable relationship between the frequency of pneumonia cases and different patients demographic information including; age, sex, source of infection (i.e., community acquired pneumonia (CAP's) or Hospital acquired pneumonia (HAP's)). Present study also aims to direct the resistance rate among both Gram-positive and Gram-negative clinical disengages from pneumonia cases including *S. pneumonia* among various hospitals of Saudi Arabia. Finally, aim of the present study is to decide the circulation of pneumonia cases among various hospitals, to examine prevalence of pneumonia cases in Hajj and Umrah seasons.

2. MATERIALS AND METHODS

2.1 Samples Collection

The study was conducted using clinical microbiology data collected at four different acute care hospitals during period of 1st Jumada al-Thani to the 30th Jumada al-Awwal of the Islamic year 1429-1430 AH (6 Jun 2008 - 25 May 2009). The study was ethically approved by the institutional review board at each of the four participating hospital prior to study commencement. Informed consent was obtained from patients or their guardians those included in sampling of this study.

2.2 Sampling Criteria

The criteria for the enrollment was all those patients with clinical diagnosis of pneumonia including; coughing with sputum, cold with shivering, fever, chest pain, shortness of breath and rapid breathing. The sputum samples submitted to the microbiology laboratory. Four hundred and forty two sputum were obtained from Al-Noor, King Faisal, King Abdul Aziz and Hera hospitals.

2.3 Sub Culture and Storage of Specimens

The organisms were sub-cultured on blood and MacConkey agar. After overnight incubation at 37ºC, they were isolated in trypticase soya broth,

containing 15% Glycerol (850 µl bacterial broth and 150 µl glycerol), further stored at -80ºC.

2.4 Automated Identification and Antimicrobial Susceptibility Testing (AST)

The study was conducted using clinical microbiology data collected at four different acute care hospitals. For the purposes of this study and consistent with Clinical and Laboratory Standards Institute (CLSI) guidelines for antimicrobial susceptibility reports, only the first organism-specific clinical culture per patient, per year, was included [7]. The method for determining susceptibility to specific antimicrobial agents varied slightly between isolates and between the four institutions, but always conformed to established national standards for laboratory practice. Laboratory methods did not vary at any hospital during the period in which data were collected for the study. In some cases, the range of pathogens or antimicrobial agents that was tested was limited for practical reasons unrelated to the conduct of the study. Bacterial isolates for which antimicrobial susceptibility results were not available for a particular agent were excluded only from the analysis of that specific agent, but remained eligible for inclusion in the analysis of other agents for which susceptibility data were available.

Preserved bacteria were sub cultured in a suitable media and incubated accordingly to obtain a pure culture. Around 3 ml of clean saline (fluid 0.45% NaCl, pH 7.0) was put into a reasonable plastic test tube. By then adequate number of morphologically comparative states of immaculate microbes was exchanged to a tube containing the saline to make homogenous suspension with an identical thickness of McFarland (No.0.50-0.63) using calibrated VITEK2 DENSI CHEK 2 system (bioMérieux, Durham, NC) [8]. The tube was then set in the tape with the recognizable proof card and information passage. To another tube holding 3 ml of saline, 280 ml of the suspension prepared for Antimicrobial susceptibility testing (AST)-GP was then transferred. The tube was then put in the anti-infection agents weakness tape, the recognizable proof GP depends on 43 biochemical tests measured carbon source usage, enzymatic actions and resistance. For the MIC (minimum inhibitory concentration) technique, AST (Antimicrobial susceptibility testing) card contains 64 micro wells were used. While control well was occupant on all card with the remaining wells containing premeasured measures of particular antimicrobials consolidated with culture medium. MIC values were determined for each antimicrobial contained on the card after a defined period of time (about 18 hrs).

2.5 Data Analysis

Data entry and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS program Ver 17.0). Descriptive statistics and Fisher's test were performed. Statistical significance was set at <0.05 to check the level of significance.

3. RESULTS

Data expressed 56%, 18%, 15% and 11% samples collected from Al-Noor hospital (ANH), King Faisal hospital (KFH), King Abdul Aziz hospital (KAH) and Hera hospital respectively. Current data showed that out of 6549 samples, 3716 samples were positive representing a proportion of 56.7% (data not shown). The difference between the total samples tested and positive samples was calculated statistically significant (p. value <0.05).

Data collected during one year expressed significant number of pneumonia cases (519, 14% of total positive) in Dull Hijjah followed by Shawwal month (389, 10.5%) (data not shown).Gender and age groups results demonstrated that the infection affects all age groups. Significant number of both suspected and positive cases were reported in the age groups of 20-65 years, followed by elderly (>65 years) people representing proportion of 51% and 36% respectively. Significantly higher ratio of pneumonia cases was observed in male gender as compared to females.

In current study, total 1,733 (98.2%) indoor while 31 (1.8%) outdoor patients were found affected to pneumonia. Results displayed that most indoor-patients infected with pneumonia were related to ICU (49.6%) ward, followed by medical wards (MW) (31.7%), surgical wards (SW) (10.6%). Out-door patients department (OPD) and emergency room (ER) contributed 64.5% and 35.5% respectively.

The study disclosed that the majority of pneumonia were HAP cases (98.2%), typically caused by *P. aeruginosa* (18.4%), *A. baumannii* (14.4%) and *S. aureus* (12.5%) followed by

K. pneumoniae (11.5%). Among the isolates causing CAP (total of 1.8%), *P. aeruginosa* (32.2%) contributed maximum followed by *K. pneumoniae* (29%) and *S. aureus* (12.9%) (Fig. 3.1). In case of gram-positive isolates higher antimicrobial sensitivity was reported for *S. aureus* followed by *S. pneumoniae* (Fig. 3.3; Table 3.1). Among gram negative isolates significantly higher antimicrobials sensitivity was reported in case of *P. aeruginosa*, *K. pneumoniae* and *A. baumannii* (Fig. 3.3; Table 3.2).

4. DISCUSSION

Pneumonia is distraction of the lungs, including the alveolar ducts and sacs, in relationship with radiographic signs [9]. Pneumonia positions sixth among the reasons for death today [10]. Clinically, there are various types of pneumonia; however a typical method for separating it is to arrange it as CAP and HAP. The merge of physical events, stuffing and any prior health conditions upgrade the exposure of getting potent illness accompanied by Hajj [11]. Because of its simplicity of transmission by RTI quicken the most widely recognized contagious infection during Hajj and Umrah seasons [12]. RTI was accounted for as the most widely recognized cause (57%) with pneumonia being the main purpose behind affirmation in 39.4% of all patients in Dull Hijjah and Umrah season [13]. Pneumonia has been most widely recognized reasons for hospitalization during Hajj, accounting 19.7% [14]. Most surprisingly higher number of pneumonia cases in Dull Hijjah month reported followed by Umrah (Ramdan) surely in light of the fact that more number of explorers originating from everywhere throughout the world because of significant chest infections, fatigue and poor nutrition among pioneers [11].

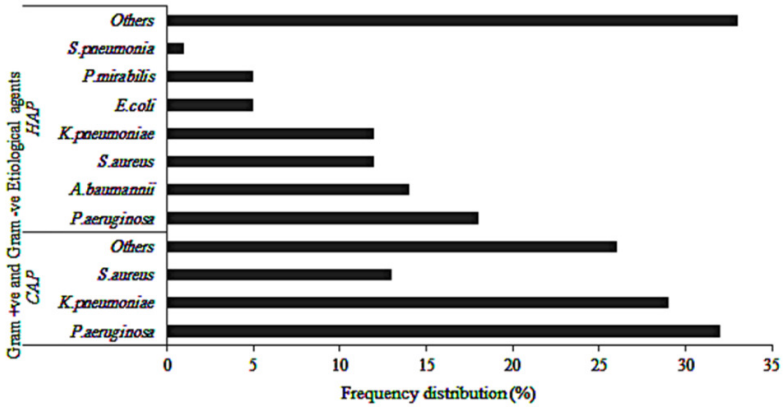

Fig. 3.1. Frequency (%) of gram +ve and gram –ve etiological agents

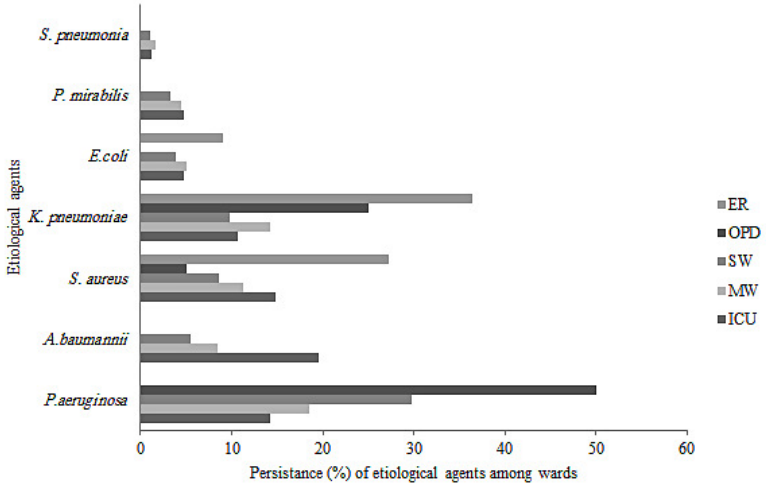

Fig. 3.2. Distribution of etiological agents among hospitals wards

Table 3.1. Resistance rates among gram-positive clinical isolates

Organism	Isolates identified	Ampicillin	Amikacin	Augmentin	Ampicillin/sulbactam	Cefazolin	Cefotaxime	Ceftriaxone	Clindamycin	Ciprofloxacin	Erythromycin	Gentamicin	Imipenem	Levofloxacin	Linezolid	Norfloxacin	Oxacillin	Penicillin-G	Rifampin	Tetracycline	Trimethoprim/sulfa	Vancomycin
Staphylococcus aureus	241%	94.7	67.3	73.5	73.9	52.2	58.6	71.1	57.7	57.1	60.8	54.1	61.4	43.3	24.4	75	65.2	94.7	30.1	55	38.5	0
		189	101	166	69	23	87	149	215	184	217	196	57	30	78	4	221	171	83	160	226	203
Streptococcus pneumoniae	23%	10.5	80	7.1	0		15.4	10	14.3	30	10.5	66.7	10.0		0		0	15	33.3	70	73.7	0
		19	5	14	2		13	10	21	10	19	9	10		7		6	20	3	10	19	13
Enterococcus faecalis	9%	0	100	50			100	100	100	62.5	83.3	100	100		20		100	50	100	71.4	50	0
		9	1	2			2	1	3	8	6	7	1		5		1	2	1	7	2	9

Each organism is presented in two rows. The top row represents its resistance in percent to that antibiotic. The 2nd row represents number of isolates tested for that specific antibiotic. Resistance equal to 100% is highlighted in (Aqua color), and equal to 0% in Purple color. The empty shaded cells (red color) indicate that susceptibility testing for that specific organism is not recommended or complete testing data was not available. Odd rows express values as %age while even rows value expressed total number

Table 3.2. Resistance rates among gram-negative clinical isolates

Organism	Isolates identified	Ampicillin	Amikacin	Augmentin	Ampicillin/sulbactam	Aztreonam	Cefepime	Cefazolin	Cefuroxime	Cefotaxime	Cefoxitin	Ceftazidime	Ceftriaxone	Cephalothin	Ciprofloxacin	Gentamicin	Imipenem	Levofloxacin	Nitrofurantoin	Piperacillin	Piperacillin-tazobactam	Tetracycline	Ticarcillin/clavulanate	Tobramycin	Trimethoprim/sulfa
Acinetobacter baumannii	270%	96.6	85.2	97.3	77.6	93.4	93.8	93.1	91.5	94.7	97.1	91.6	94.3	96.9	94.2	80.9	70.5	82.1	92	94.7	92.4	79.7	91.7	64.6	88.1
		177	250	150	107	181	225	58	118	226	170	251	123	162	241	241	234	95	50	247	158	212	84	113	236
Pseudomonas aeruginosa	371%	67	38.2	65.3	38.5	49.7	47.2	39.5	65.8	74.2	68.5	49.2	68	68.1	43.3	47	29.2	51.6	36.6	51.7	36.7	76.7	50	40.5	68.6
		88	343	75	39	147	318	43	73	168	89	307	128	91	312	332	319	95	41	300	245	73	102	131	105
Klebsiella pneumoniae	238%	94.3	23.4	54.3	64.6	63.9	57.5	70	64.5	59.2	45.6	58.3	59.8	72.9	56.3	50.5	13.1	55.2	64.1	74.9	38.3	62.2	48.3	62.8	57.4
		212	222	175	65	122	200	60	169	174	160	192	102	207	208	218	199	58	39	175	175	143	58	78	209
Klebsiella oxytoca	11%	77.8	0	60	50	83.3	33.3	100	42.9	37.5	66.7	44.4	66.7	87.5	37.5	12.5	11.1	50	50	75	11.1	42.9	50	100	50
		9	8	5	2	6	9	2	7	8	6	9	3	8	8	8	9	2	2	8	9	7	2	2	8
Escherichia coli	94%	96.4	22.2	73.7	85.7	72.3	68.5	85.7	81.1	71.4	42.9	68.6	65.2	91.7	70	63.6	8	100	60	88.2	39.3	80	44.4	90.9	78.5
		84	90	76	7	65	73	7	53	70	77	86	23	84	80	88	87	6	10	76	61	50	9	11	79
Proteus mirabilis	93%	96.1	77	79.2	75	48.5	59.1	83.3	87.5	76.6	75.7	72	63	89.2	84.6	91.7	20	81.8	81.8	93.3	36.1	100	15.4	73.3	93.1
		77	87	77	12	68	88	12	56	77	74	82	27	83	78	84	85	11	11	75	72	53	22	15	87
Enterobacter cloacae	18%	93.3	23.5	91.7	80	72.7	40	100	87.5	80	100	76.5	75	100	17.6	41.2	11.8	40	100	86.7	60	50	80	50	43.8
		15	17	12	5	11	15	5	16	15	11	17	8	17	17	17	17	5	1	15	15	12	5	6	16
Enterobacter aerogenes	10%	90	60	100	75	50	70	100	71.4	71.4	60	77.8	83.3	88.9	66.7	60.0	22.2	40	100	87.5	22.2	85.7	75%	83.3	80
		10	10	5	4	4	10	4	7	7	5	9	6	9	9	10	9	5	2	8	9	7	4	6	10
Haemophilus influenzae	12%	36.4	14.3	10		40	25	0	0	14.3	12.5	20	0	45.5	25	16.7	16.7			75	100	66.7			90.9
		11	7	10		5	4	1	5	7	8	5	1	11	8	6	6			4	1	3			11

Serratia marcescens	29%	96.4	25	100	83.3	53.3	48.1	90.9	95.7	60	77.8	50	60	96.2	42.3	42.9	19.2	58.3	75	65.2	38.1	68.2	40	61.5	44.8
		28	28	19	12	15	27	11	23	25	18	28	15	26	26	28	26	12	4	23	21	22	10	13	29
Providencia stuartii	29%	96.6	70.4	100	85	66.7	74.1	100	88.5	76	42.9	75	76.2	100	85.7	92.9	14.8	68.4	100	44.4	23.1	100	15.8	95.2	82.6
		29	27	7	20	6	27	20	26	25	7	28	21	27	28	28	27	19	2	27	26	26	19	21	23
Citrobacter	8%	62.5	50	100	100	66.6	50	100	100	50	100	50	100	83.3	100	25	25	100	100	100	50	100	0	100	83.3
Species		5	2	2	1	3	2	1	2	2	2	4	1	6	4	2	4	1	1	4	2	2	1	1	6
Morganella morganii	12%	100	58.3	100	57.1	100	36.4	100	75	40	50	45.5	40	100	58.3	90	12.5	57.1	100	63.6	18.2	100	28.6	88.9	91.7
		12	12	6	7	1	11	7	8	10	2	11	10	11	12	10	8	7	1	11	11	10	7	9	12
Stenotropho-monas maltophilia	13%	100	100	100	100	100	100	100	100	100	100	69.2	100	100	80	100	100	60	100	80	100	85.7	66.7	100	8.3
	%	8	8	5	2	9	6	2	5	6	6	13	2	8	10	9	10	5	1	10	7	7	3	3	12

Each organism is presented in two rows. The top row represents its resistance in percent to that antibiotic. The 2nd row represents number of isolates tested for that specific antibiotic. Resistance equal to 100% is highlighted in (Aqua color), and equal to 0% in Purple color. The empty shaded cells (red color) indicate that susceptibility testing for that specific organism is not recommended or complete testing data was not available. Odd rows express values as %age while even rows value expressed total number

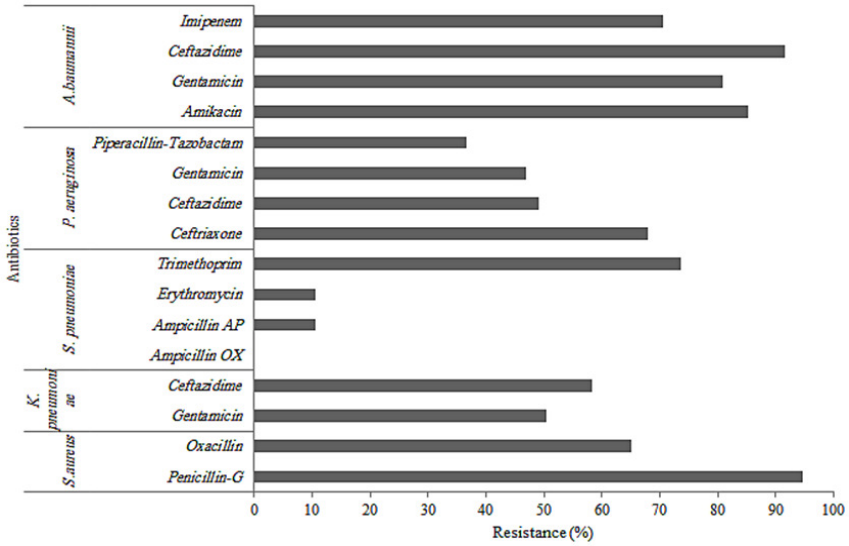

Fig. 3.3. Antibiotics resistance (%) on different etiological agaents causing pneumonia

Among the four hospitals incorporated into this study, results demonstrated that ANH had more than half of aggregate sputum tests submitted for testing. Present finding is most likely because of two components: the first is the higher number of beds in ANH in contrast with different hospitals, and another reason might be the physical closeness of ANH to Mena, Arafat and Muzdalifa. Positive samples (56.7%) with a momentous gap between the aggregate specimens tried were highlighted at cutoff P-value <0.05. This vast gap could be because of a few variables including clinical misdiagnosis of cases, antimicrobial use, lacking method of sputum sampling and infrequent pneumonia microbes that are not routinely investigated.

In the present study, the occurrence of pneumonia in male was higher than in females (1.8:1) potentially on account of the distinctions in the societal position between the two genders. Current finding follows with those already reported in countries like Iraq, USA, Thailand and Saudi Arabia [15-17]. A comparative finding was seen during month of Dull Hijjah, where the proportion of disease in the middle of male and female was 1.6:1. Present study shows 98.2% indoor-patients and 1.8% outdoor patients which is in contrast to previous studies, both at neighborhood [12] and global levels [16-17]. In current study, greater part of HAP cases were mainly contributed by *P. aeruginosa* (18.4%) and *A. baumannii* (14.4%) followed by *S. aureus* (12.5%) and *K. pneumoniae* (11.5%). Current results follow similar studies done national and global level which also reported that most HAP cases brought about by Gram-negative microorganisms [18-19].

In present study *P. aeruginosa* (32.2%), *K. pneumoniae* (29%) and *S. aureus* (12.9%) were observed to be most basic causative operators for pneumoniae among different hospital wards. Current findings are also supported by previous studies which also reported that *S. pneumoniae* is the most frequent microorganism for CAP [19-20]. This may be due to mishandling of observational treatment given to out-patients before taking sputum tests for microbiological refined.*S. aureus* (17.1%), *A. baumannii* (15.5%), *K. pneumonia* (14.5%) and *P. aeruginosa* (12.8%) are basic microbes found in Dull-Hajj season.The conclusion of pneumonia was confirmed in 72% patients. *M. tuberculosis* (28%) and Gram-negative (26%) were among the most widely recognized isolates. *S. pneumoniae* was distinguished in 10% patients while infrequent isolates were recognized in 6% patients. Past studies reported that the etiological operators of pneumonia in Hajj differed from those of CAP [11]. Most persistent pathogens causing *S. pneumoniae* were probably due to *H. influenza*, *K. pneumoniae*, and *S. pneumoniae* [21].

Most isolates in this study are multidrug resistant. This may be attributed to probability of the occurrence of genes that code for multiple antibiotics resistance in the isolates. Some of the

ways by which bacteria can develop antibiotics resistance include: (i) Antibiotic inactivation – direct inactivation of the active antibiotic molecule [22]; (ii) Target modification – alteration of the sensitivity to the antibiotic by modification of the target [23]; (iii) Efflux pumps and outer membrane (OM) permeability changes–reduction of the concentration of drug without modification of the compound itself [24]; or (iv) Target bypass – some bacteria become refractory to specific antibiotics by bypassing the inactivation of a given enzyme. Resistance could be developed to antibiotics by variation in medication target proteins and adaptations that limit the absorption of the drug to the target [25].

5. CONCLUSION

This study provides information on the most common pneumonia causes, and the demographic distribution of the disease. Our study outcomes will aid health care planners, administrators, and officials in order to provide optimal health care services to locals in general and pilgrims during Hajj and Umrah seasons. Clinicians should be aware that typical pneumonia treatment regimes may not work well due to the wide variety of isolated organisms. This necessitates taking a sputum sample before starting treatment for identification and sensitivity testing since a single dose of antibiotic treatment prior to pneumonia diagnosis may hinder the detection of the causative agent.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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