Indian Journal of Applied Microbiology
ISSN (Online): 2454-289X, ISSN (Print): 2249-8400

Volume 21 Number 2
July - September 2018, pp. 1-10

Prevalence and Drug resistance pattern of *Klebsiella pneumoniae* causing Community - Acquired Pneumonia in Paonta Sahib Region of Himachal Pradesh

Mony Thakur[*1, 2] and Khushboo[3]

[1]Dept. of Microbiology, Himachal Institute of Life Sciences, Paonta Sahib (H.P.), India
[2]Dept. of Microbiology, Central University of Haryana, Jant-Pali, Mahendergarh, India
[3]Dept. of Biotechnology, Central University of Haryana, Jant-Pali, Mahendergarh, India

Abstract: Bacteria belonging to the genus Klebsiella frequently cause human community-acquired infections. Medically most important klebsiella species *K. pneumoniae* accounts for a significant proportion of community-acquired pneumonia (CAP). *K. pneumoniae* has been associated with different type of infections and one of the most important aspects of Klebsiella is the emergence of MDR strains particularly in clinical isolates. The study was carried out from June 2013 to June 2014. A total of 106 sputum samples were collected from patients in Civil Hospital, Dr. Puran Chand hospital and Sidhivinayak Hospital in Paonta Sahib followed by the biochemical characterization recovered isolates are *K. pneumoniae* (n=27; 25.4%), *S. pneumoniae* (n=40; 37.7%), *Pseudomonas* (n=20; 18.8%). All recovered isolates of *Klebsiella pneumoniae* were resistant against amikacin, amoxicillin, ampicillin, aztreonam, bacitracin, cefixime, chloramphenicol, cloxacillin, ciprofloxacin, kanamycin, meropenem, oxacillin, streptomycin, and tetracycline while they were intermediate against gentamycin.

Keywords: CAP, MDR, RTI, Antimicrobial resistance.

Introduction:

The developing countries are facing number of problems related to lower respiratory tract infections (LRTI) which in turn are responsible for desolation and mortality in the children aged ≤ 5 years [16]. Community-Acquired Pneumonia (CAP) is a frequently caused pneumonia revealing

*Author for Correspondence. E-mail: mony.thakur0807@gmail.com

about 1/1000 of the adult domain of society per year. This pneumonia crops up when the bacteria attack the alveolar spaces of the respiratory tract leading an inflammatory reaction which escorts to the clinical aspects like cough, spittle production, problem in breathing and sporadically chest pain [23]. Community Acquired Pneumonia (CAP) can be provoked by immense form of bacteria that emanate from the patient's surroundings like *S. pneumoniae, M. pneumoniae, H. influenza, K. pneumoniae* etc. These bacteria along with liability of dejection elucidate the period of hospitalization, wide range of antimicrobial agents and also intensify the health care expense [17-22].

Among the neonates admitted to the hospitals in many countries, multidrug resistant gram negative bacilli belonging to family Enterobacteriaceae is increasingly responsible in which *Klebsiella* sp. constitute the majority of these pathogens [2]. It is more common in males and persons who are diabetic, alcoholic or hospitalized, more sensitive to pneumonia. Infection caused by *Klebsiella pneumoniae is* distinguishable from other bacterial pneumonia, except the severe infection and confluent pneumonia of lobular distribution caused by other gram negative bacteria. Cavitations and abscess formation may occur with Staphylococcal pneumonia although these tend to be less widespread. Sputum is viscid like redcurrant jelly and may be blood stained. Inadequate and prolonged antimicrobial prophylaxis increases resistance to antimicrobial drugs [3]. The term multi drug resistance (MDR), which initially describes resistant mammalian tumor cells and later strains of *M. tuberculosis*, now describes multi drug resistance in any micro organism – bacterium, fungus or parasite. *Klebsiella pneumoniae* is among the most common gram-negative bacteria known medically to be an important pathogenic bacterium that is opportunistic in nature [4]. Mortality may be as high as 14% overall whereas, for the elder peoples mortality reaches greater than 50% within 5 years [5]. Developing countries particularly in sub-Saharan Africa, financial problems affecting proper antibiotic treatment of infectious disease poses an immense threat to their health systems, because of rapidly spreading antibiotic resistance [6]. The manifestation and expansion of antibiotic resistance in CAP infections is an awful situation at the global scale and had an extensive conflict on antimicrobial management, touching all age group [24-27].

Materials and Methods:

Sample Collection

During the study period, sputum samples from patients admitted with pneumonia were collected from Dr. Puran Chand hospital, Sidhivinayak hospital, and Civil hospital at Paonta Sahib (H.P). The samples were collected aseptically in sterile 50 ml Oakridge tubes containing 0.85% saline solution. These samples were taken up by medical practitioners and then collected from the laboratories of concerned hospitals and clinics for further assessment. The samples received were initially inoculated in nutrient broth for the enrichment of sample. The little amount of enriched culture picked up using inoculating loop was streaked (quadrant) over Mac Conkey agar for the selective isolation of *Klebsiella pneumoniae.*

Isolation and Identification of Bacterial strains:

Clinical isolates of *Klebsiella pneumoniae* was collected from the different places of Paonta Sahib Region in Himachal Pradesh. All recovered isolates were identified on the basis of colony morphology on MacConkey agar media. Isolates showing mucoid colonies were further examined using standard biochemical tests *viz,* catalase, oxidase, citrate utilization, nitrate reduction & urease production.

Antibiotic sensitivity test:

The prevalence of resistance pattern of bacteria causing pneumonia disease was detected by Kirby Bauer disc diffusion method, on Muller Hinton Agar [7]. 100μl of each bacterial suspension of different isolates uniformly spread over respective MHA (Muller-Hilton agar) plates and allowed to dry before applying antibiotic discs. Then, commercially available antibiotic discs (Hi-Media, India) were placed over inoculated media and pressed gently followed by the 24 hr. incubation period. Antibiotics that were used are amikacin (30mcg), amoxicillin (25mcg), ampicillin (10mcg), aztreonam (30mcg), bacitracin (8units), cefixime (5mcg), chloramphenicol (30mcg), cloxacillin (5mcg), ciprofloxacin (5mcg), kanamycin (5mcg), meropenem (10mcg), oxacillin (1mcg), streptomycin (10mcg), tetracycline (30mcg), and gentamycin (50mcg). Antimicrobial activity of these antibiotic discs is indicated by a zone of inhibition which can be measured to check the susceptibility pattern. Susceptibility of bacterial strains to these antibiotics is categorized into three categories on the basis of the diameter of inhibition zone, Resistance (<13mm), Intermediate (15-18mm), and Sensitive if the diameter was more than 19mm.

Results:

Bacterial isolation:

A total of 87 isolates were recovered from 106 clinical samples collected from different hospitals, screened for the isolation of *Klebsiella pneumoniae.* The overall prevalence of *Klebsiella pneumoniae* was observed to be 25.4% (27/87). Out of which 07 from throat swab and 20 from sputum samples of the patients were isolated. The age wise distribution of *Klebsiella pneumoniae* infection is depicted in the table 1.3. It clearly indicates that age group of 35 years and above are more susceptible to Klebsiella infection (n=13, 40.6%).

Table 1.1 Number of samples collected

S. No	Hospital	No. of sample
1.	Dr. Puran Chand Hospital	25
2.	Civil Hospital	50
3.	Sidhivinayak Hospital	31

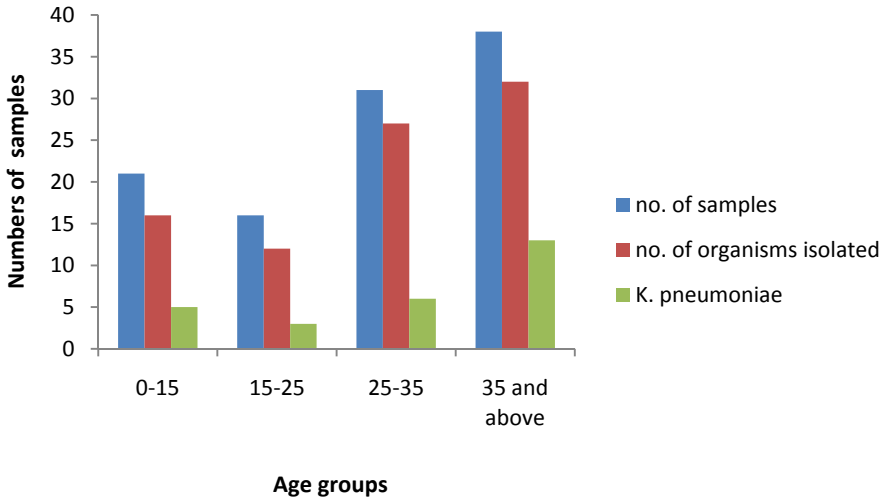
Table 1.2 No. and % of organism isolated from sputum samples

S. No.	Organism	No. of organism (n=87)	% organism
1.	*S. pneumoniae*	40	45.9
2.	*K. pneumoniae*	27	31.03
3.	*P. aeruginosa*	20	22.98

Table 1.3 Incidence of *K. pneumoniae* infection in individuals of different age group

Age group (In years)	No. of samples (N)	Isolated organism (n)	Recovered isolates of *K. pneumoniae*	Prevalence %
0-15	21	16	05	31.25
15-25	16	12	03	25
25-35	31	27	06	22.2
35 and above	38	32	13	40.62
Total	N=106	n=87	27	

Graph 1: Prevalence of K. pneumoniae in different age groups



Antimicrobial susceptibility test:

Susceptibility tests results by disc diffusion method were depicted in table 1.4. The recovered isolates of *K. pneumoniae* showed high resistance pattern. They were resistant to the different antibiotics like amikacin (59.2%), amoxicillin (77.75), ampicillin (88.8%), aztreonam (100%), bacitracin (100%), cefixime (85.1%), chloramphenicol (57.8%), cloxacillin (100%), ciprofloxacin (85.1%), kanamycin (100%), meropenem (100%), oxacillin (100%), streptomycin (77.7%), tetracycline (100%) and gentamycin found to be sensitive (25.9%) among the all tested antibiotics.

Table 5.3 Zone of inhibition diameter shown by different antibiotics

	Inhibition Zone Diameter (mm)														
Isolate No.	AK	AM	AMP	AO	B	CFM	C	COX	CIP	GEN	K	MRP	OX	S	TE
S001	12(I)	14(I)	N.Z	9(R)	6(R)	11(I)	16(S)	5(R)	10(R)	18(S)	5(R)	6(R)	9(R)	14(I)	8(R)
S002	9(R)	11(I)	N.Z	N.Z	8(R)	6(R)	N.Z	3(R)	N.Z	17(I)	8(R)	8(R)	N.Z	11(I)	9(R)
S003	15(I)	5(R)	N.Z	7(R)	N.Z	N.Z	12(I)	9(R)	14(I)	20(S)	4(R)	3(R)	7(R)	5(R)	N.Z
S004	9(R)	N.Z	6(R)	N.Z	N.Z	8(R)	N.Z	N.Z	N.Z	15(I)	N.Z	N.Z	N.Z	N.Z	N.Z
S005	7(R)	4(R)	14(I)	3(R)	3(R)	14(I)	16(S)	9(R)	5(R)	13(I)	8(R)	7(R)	3(R)	4(R)	N.Z
S006	14(I)	N.Z	3(R)	N.Z	N.Z	N.Z	4(R)	2(R)	N.Z	15(I)	N.Z	N.Z	N.Z	N.Z	N.Z
S007	12(I)	15(I)	7(R)	N.Z	N.Z	4(R)	N.Z	7(R)	N.Z	14(I)	4(R)	7(R)	N.Z	15(I)	5(R)
S008	N.Z	8(R)	16(I)	N.Z	4(R)	8(R)	18(S)	5(R)	14(I)	19(S)	5(R)	N.Z	N.Z	8(R)	N.Z
S009	N.Z	N.Z	N.Z	4(R)	N.Z	N.Z	N.Z	N.Z	N.Z	12(I)	N.Z	4(R)	4(R)	N.Z	N.Z
S010	9(R)	16(I)	8(R)	7(R)	N.Z	N.Z	9(R)	4(R)	N.Z	13(I)	N.Z	N.Z	7(R)	16(I)	N.Z
S011	13(I)	N.Z	7(R)	8(R)	7(R)	N.Z	15(I)	9(R)	8(R)	22(S)	9(R)	4(R)	8(R)	N.Z	8(R)
S012	8(R)	4(R)	N.Z	N.Z	N.Z	13(I)	14(I)	7(R)	12(I)	18(S)	8(R)	N.Z	N.Z	4(R)	N.Z
S013	16(I)	N.Z	13(I)	9(R)	6(R)	4(R)	20(S)	9(R)	N.Z	14(I)	8(R)	7(R)	9(R)	N.Z	N.Z
S014	7(R)	8(R)	4(R)	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	17(I)	N.Z	8(R)	N.Z	8(R)	5(R)
S015	N.Z	N.Z	N.Z	N.Z	N.Z	11(I)	13(I)	8(R)	8(R)	13(I)	6(R)	N.Z	N.Z	N.Z	N.Z
S016	6(R)	11(I)	8(R)	N.Z	N.Z	N.Z	N.Z	N.Z	6(R)	12(I)	N.Z	9(R)	N.Z	11(I)	9(R)
S017	13(I)	N.Z	4(R)	N.Z	N.Z	8(R)	6(R)	9(R)	N.Z	14(I)	3(R)	N.Z	N.Z	N.Z	N.Z
S018	5(R)	9(R)	N.Z	N.Z	3(R)	N.Z	N.Z	N.Z	3(R)	16(I)	N.Z	4(R)	N.Z	9(R)	4(R)
S019	9(R)	N.Z	11(R)	N.Z	8(R)	5(R)	12(I)	8(R)	N.Z	13(I)	6(R)	N.Z	N.Z	N.Z	N.Z
S020	4(R)	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	7(R)	12(I)	N.Z	4(R)	N.Z	N.Z	N.Z
S021	15(I)	14(I)	10(R)	N.Z	5(R)	2(R)	17(S)	N.Z	16(I)	18(S)	7(R)	5(R)	N.Z	14(I)	8(R)
S022	9(R)	4(R)	N.Z	N.Z	N.Z	N.Z	N.Z	6(R)	N.Z	16(I)	N.Z	8(R)	N.Z	4(R)	N.Z
S023	14(I)	N.Z	N.Z	4(R)	7(R)	5(R)	15(I)	4(R)	N.Z	14(I)	N.Z	6(R)	4(R)	N.Z	6(R)
S024	6(R)	N.Z	N.Z	N.Z	N.Z	N.Z	8(R)	N.Z	6(R)	12(I)	N.Z	3(R)	N.Z	N.Z	N.Z
S025	13(I)	6(R)	5(R)	5(R)	9(R)	N.Z	14(I)	9(R)	N.Z	18(S)	9(R)	6(R)	5(R)	6(R)	4(R)
S026	N.Z	N.Z	3(R)	N.Z	N.Z	7(R)	N.Z	N.Z	3(R)	11(I)	N.Z	N.Z	N.Z	N.Z	N.Z
S027	14(I)	N.Z	N.Z	N.Z	7(R)	N.Z	13(I)	6(R)	7(R)	14(I)	N.Z	N.Z	N.Z	N.Z	6(R)

I- Intermediate, S- Sensitive, R-Resistance, N.Z - No zone of inhibition

Table 1.4 Percentage of antibiotics susceptibility of *Klebsiella pneumoniae*

Antibiotics	Sensitivity (%)	Intermediate (%)	Resistance (%)
Penicillin			
AMP	0	11.1	88.8
AM	0	22.2	77.7
COX	0	0	100
OX	0	0	100
Mono-bactam			
AO	0	0	100
Tetracyclines			
TE	0	0	100
Aminoglycosides			
AK	0	40.7	59.2
GEN	25.9	74.0	0
K	0	0	100
S	0	22.2	77.7
Carbapenem			
MRP	0	0	100
Cephalosporin			
CFM	0	14.8	85.1
Fluoroquinolones			
CIP	0	14.8	85.1
Other anti-bacterial			
B	0	0	100
C	18.5	29.6	57.8

AK=Amikacin; AM=Amoxicillin; AMP=Ampicillin; AO=Aztreonam;
B=Bacitracin; CFM=Cefixime; C=Chloramphenicol; COX=Cloxacillin;
CIP=Ciprofloxacin; GEN=Gentamycin; K=Kanamycin; MRP=Meropenem;
OX=Oxacillin; S=Streptomycin; TE=Tetracycline;

Discussion:

This study was carried out to examine the susceptibility of *K. pneumoniae* isolates collected from sputum of different patients toward 15 different antibiotics. Infections caused by gram negative bacteria are a major matter of concern. In our study the most prominent bacteria found to be

involved in community acquired pneumoniae is *S. pneumoniae* (37.7%) followed by the *K. pneumoniae* (25.4%). *K. pneumoniae* can cause a classic form of primary pneumoniae. It is found in the oropharynx of a normal person. However prevalence rate is high in hospitalized patients. A study conducted by WHO (World Health Organization) estimates 1.6 million deaths annually in adults aged over 59 years. In India 89.5 deaths/ 100,000 populations was due to lower respiratory tract infections, while 62.0 in United Kingdom and 21.3 in United States of America [8]. Factors like alcoholism, bronchial asthma, age >70 years, compromised immune system and smoking influence the type of pathogens that should be considered in identifying the etiologic agent [9]. Our study showed the total percentage of positive cases as 25.4%. The large gap between the number of samples collected and positivity could be due to clinical misdiagnosis of cases, pre-antibiotic usage and inadequate sputum collection technique. The higher incidence of infections due to *K. pneumoniae* during the past decade probably reflects an increase in community-acquired pneumoniae in immune suppressed individuals and continuous increase in the antibiotic resistance. In an epidemiological survey of individual's aged between 15–64 years, an incremental annual incidence rate was observed (1.12–3.16 per 1000 inhabitants) [9]. In Udupi, community based study recorded 6.42 episodes per child/year with incidence of ARI, out of which 8.7% (51 of 584 episodes) were pneumonia and 3 ARI episodes (0.5%) were severe pneumonia [10]. At three different study sites incidence of physician-diagnosed pneumonia at discharge per child per year was 0.030 (95% CI 0.025-0.034) at Chandigarh, 0.080 (95% CI 0.071-0.091) at Kolkata and 0.037 (95% CI 0.030-0.045) at Vellore. Age-specific incidence of severe pneumonia in infants <5 months of age was highest and decreases with increasing age [11]. Mortality in urban areas comprises 24.9% deaths and 28.0% deaths in rural areas due to pneumonia. Highest deaths rate due to pneumonia were recorded in north India (Jammu & Kashmir, Delhi) and lowest in south India (Tamil Nadu). From the collected data of annual deaths in our whole country it is estimated that 13·5% (99% CI 13·0-14·1) mortality due to pneumonia [12]. 736 positive cultures (22.4%) of Gram-negative *Klebsiella pneumoniae* out of 4027 samples from children <15 years of age showed 70-80% resistance to amoxicillin and cephalexin, and minimum resistance to cefotaxime (23%) and ciprofloxacin (12%) [13]. A study conducted in Shimla (Himachal Pradesh) showed isolation rates for *Klebsiella pneumoniae as* (n=12), 22.6% [14]. When compared with our study in Paonta Sahib Region of Himachal Pradesh isolation rates were 25.4% (27/87). According to the study conducted in Andhra Pradesh [15] recovered isolates of *Klebsiella pneumoniae* from sputum samples were 33.3% (2008), 38.88% (2009), 43.08% (2010) resistant against amikacin (AK) and in our study recovered isolates of *Klebsiella pneumoniae* shows 59.2 % (n=16) (2013-2014) resistance to AK and 51.85% (2008) against tetracycline 54.17% (2009) and 60% (2010) of them are 100% (n=27) resistant.

Thus this study provided information regarding the prevalence and the drug resistance pattern of the recovered isolates. This data will help health care hospitals to provide appropriate health care services to the patients' suffering from community acquired pneumonia. As most of the patients were 35 years old and above, special precautions should be taken with this age group, including immunization, avoiding crowds and using facial masks to reduce the chance of infection. The majority of the isolates were sensitive towards gentamycin. The increase in resistance and gradual decrease in sensitivity was observed for all the antibiotics tested against *K. pneumoniae* strains

isolated from the sputum sample. An attempt has been made in this study to recognize the prevalence and record the antibiogram of bacteria in Paonta Sahib Region of Himachal Pradesh.

Acknowledgements:

I am thankful to all consultants and the lab attendees of Dept. of Microbiology of Himachal Institute of Life Sciences, Paonta Sahib who supported and guided in this work. I thank Dr. Puran Chand hospital, Sidhivinayak hospital, and Civil hospital for their cooperation. Special thanks to Dr. V.K Gupta (Chairman), Himachal Institute of Life Sciences, for his timely help and encouragement during the study period.

Conflict of Interest

There was no conflict of interest.

References

1. Brar, N.K. and Niederman, M.S., 2011, Management of community-acquired pneumonia: a review and update. *Therapeutic advances in respiratory disease*, *5*(1), pp.61-78.
2. Baby padmini, S. and Appalaraju, B., 2004. Extended spectrum-lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*-prevalence and susceptibility pattern in a tertiary care hospital. *Indian Journal of Medical Microbiology*, *22*(3), p.172.
3. Okeke, I.N., Laxminarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., Pablos-Mendez, A. and Klugman, K.P., 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. *The Lancet infectious diseases*, *5*(8), pp.481-493.
4. Ko, W.C., Paterson, D.L., Sagnimeni, A.J., Hansen, D.S., Von Gottberg, A., Mohapatra, S., Casellas, J.M., Goossens, H., Mulazimoglu, L., Trenholme, G. and Klugman, K.P., 2002. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. *Emerging infectious diseases*, *8*(2), p.160.
5. Ewig, S. and Torres, A., 2011. Community-acquired pneumonia as an emergency: time for an aggressive intervention to lower mortality. *European Respiratory Journal*, *38*(2), pp.253-260.
6. Alabi, A.S., Frielinghaus, L., Kaba, H., Kösters, K., Huson, M.A., Kahl, B.C., Peters, G., Grobusch, M.P., Issifou, S., Kremsner, P.G. and Schaumburg, F., 2013. Retrospective analysis of antimicrobial resistance and bacterial spectrum of infection in Gabon, Central Africa. *BMC infectious diseases*, *13*(1), p.455.
7. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, *45*(4), p.493.
8. The global burden of the disease. 2004 update. Available at: http://www.who.int/healthinfo/global burden disease/GBD report 2004 update.

9. Almirall, J., Bolibar, I., Vidal, J., Sauca, G., Coll, P., Niklasson, B., Bartolome, M. and Balanzo, X., 2000. Epidemiology of community-acquired pneumonia in adults: a population-based study. *European Respiratory Journal*, *15*(4), pp.757-763.

10. Acharya, D., Prasanna, K.S., Nair, S. and Rao, R.S., 2003. Acute respiratory infections in children: a community based longitudinal study in south India. *Indian journal of public health*, *47*(1), pp.7-13.

11. Gupta, M., Kumar, R., Deb, A.K., Bhattacharya, S.K., Bose, A., John, J., Balraj, V., Ganguly, N.K., Kant, L., Kapoor, A.N. and Watt, J., 2010. Multi-center surveillance for pneumonia & meningitis among children (< 2 yr) for Hib vaccine probe trial preparation in India.

12. Million Death Study Collaborators, 2010. Causes of neonatal and child mortality in India: a nationally representative mortality survey. *The Lancet*, *376*(9755), pp.1853-1860.

13. Chaudhary, M., Kumar, S. and Payasi, A., 2013. Prevalence and antimicrobial sensitivity of extended spectrum beta-lactamase producing Gram negative bacteria from clinical settings in India from 2010-2012. *Int J Med Sci*, *46*, pp.1212-1217.

14. Bansal, S., Kashyap, S., Pal, L.S. and Goel, A., 2004. Clinical and bacteriological profile of community acquired pneumonia in Shimla, Himachal Pradesh. *Indian Journal of Chest Diseases and Allied Sciences*, *46*(1), pp.17-22.

15. Sarathbabu, R., Ramani, T.V., Rao, K.B. and Panda, S., 2012. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. *IOSR J Pharm Biol Sci*, *1*(2), pp.04-09.

16. Madhi, S.A., Petersen, K., Madhi, A., Khoosal, M. and Klugman, K.P., 2000. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. *Clinical Infectious Diseases*, *31*(1), pp.170-176.

17. Masterton, R.G., Galloway, A., French, G., Street, M., Armstrong, J., Brown, E., Cleverley, J., Dilworth, P., Fry, C., Gascoigne, A.D. and Knox, A., 2008. Guidelines for the management of hospital-acquired pneumonia in the UK: report of the working party on hospital-acquired pneumonia of the British Society for Antimicrobial Chemotherapy. *Journal of Antimicrobial Chemotherapy*, *62*(1), pp.5-34

18. Abrahamian, F.M., DeBlieux, P.M., Emerman, C.L., Kollef, M.H., Kupersmith, E., Leeper, K.V., Paterson, D.L. and Shorr, A.F., 2008. Health care–associated pneumonia: identification and initial management in the ED. *The American journal of emergency medicine*, *26*(6), pp.1-11.

19. Foglia, E., Meier, M.D. and Elward, A., 2007. Ventilator-associated pneumonia in neonatal and pediatric intensive care unit patients. *Clinical microbiology reviews*, *20*(3), pp.409-425.

20. Masterton, R., Craven, D., Rello, J., Struelens, M., Frimodt-Moller, N., Chastre, J., Ortqvist, A., Cornaglia, G., Lode, H., Giamarellou, H. and Bonten, M.J.M., 2007. Hospital-acquired pneumonia guidelines in Europe: a review of their status and future development. *Journal of Antimicrobial Chemotherapy*, *60*(2), pp.206-213.

21. Rello, J., 2004. Bench-to-bedside review: Therapeutic options and issues in the management of ventilator-associated bacterial pneumonia. *Critical Care*, *9*(3), p.259.

22. Vidaur, L., Sirgo, G., Rodríguez, A.H. and Rello, J., 2005. Clinical approach to the patient with suspected ventilator-associated pneumonia. *Respiratory care*, *50*(7), pp.965-974.

23. Woodhead, M., 2002. Community-acquired pneumonia in Europe: causative pathogens and resistance patterns. *European Respiratory Journal*, *20*(36 suppl.), pp.20s-27s.

24. Felmingham, D., Feldman, C., Hryniewicz, W., Klugman, K., Kohno, S., Low, D.E., Mendes, C. and Rodloff, A.C., 2002. Surveillance of resistance in bacteria causing community-acquired respiratory tract infections. *Clinical Microbiology and Infection*, *8*, pp.12-42.

25. Garau, J., 2001. Clinical failures: the tip of the iceberg. *Respiratory medicine*, *95*, pp.S5-S11.

26. Cartwright, K., 2002. Pneumococcal disease in Western Europe: burden of disease, antibiotic resistance and management. *European journal of pediatrics*, *161*(4), pp.188-195.

27. Bonomo, R.A., 2002. Resistant pathogens in respiratory tract infections in older people. *Journal of the American Geriatrics Society*, *50*(7), pp.236-241.