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RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF CLINICAL CHARACTERISTICS AND ANTIBA CTERIAL SUSCEPTIBILITY BETWEEN *KLEBSIELLA PNEUMONIAE* AND *KLEBSIELLAOXITOCA* AT A TERTIARY CARE UNIVERSITY HOSPITAL IN THE CENTRAL REGION OF JAPAN FROM 2008 TO 2010

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ABSTRACT

Klebsiella species including *Klebsiella pneumoniae* and *Klebsiella oxitoca* are one of the most common pathogen bacteria that cause a variety of infections. This study was conducted to find out the prevalence and antibacterial susceptibility patterns of *Klebsiella* species isolates at tertiary care university hospital in the central region of Japan from 2008 to 2010. *Klebsiella* species was identified by standard laboratory procedure. Antibacterial susceptibility testing was performed by micro dilution assay according to CLSI recommendation. Of seven hundred ten *Klebsiella* species, five hundred fifty*Klebsiellapneumoniae*, and one hundred sixty *Klebsiellaoxitoca* were isolated. About four hundred seventy *Klebsiella* species isolates were from inpatient. The major source of *Klebsiella* isolates were sputum, and urine. Positive samples were received mostly from the urology, paediatrics, respiratory medicine, intensive care unit and surgery. The effective antibiotics with over 99% susceptibility rates were amikacin, imipenem, and meropenem. The numbers of extended-spectrum beta-lactamase (ESBL) producing isolates were thirty-five and eighteen *Klebsiella* species isolates had multidrug resistant ability. *Klebsiella* species infection spreads among community easily and inappropriate use of antibiotics contributes to their resistance. Continuous antibacterial susceptible surveys are indispensable to reduce the emergency of ESBL and multidrug resistance.

INTRODUCTION

Klebsiella species including *Klebsiella pneumoniae* and *Klebsiellaoxitoca*are one of the most common pathogen bacteria that cause a variety of infections such as pneumoniae, urinary tract and blood stream infections (Podschun R, *et al*, 1998). Furthermore, a distinctive invasive syndrome with pyogenic liver abscess formation has emerged from Taiwan in 1980s (Liu Yc. *et al*., 1986). The invasive syndrome was subsequently reported in many Southeast Asian countries (Yeh KM, *et al*, 2007)(Lok KH, *et al*, 2008). Antibacterial resistant*Klebsiella* species has been reported increasingly (Broberg CA, *et al*, 2014). The use of beta-lactams has become difficult in recent years as various classes of beta-lactamases have found in clinical *Klebsiella* isolates. The incidence of carbapenem resistance in *Klebsiella* is rapidly increasing, rising from 1.6% to 10.4% between 2001 and 2011(Broberg CA, *et al*, 2014).Although the investigation of antibiotics-resistant *Klebsiella* has been performed, little is known of the comparative analysisbetween *Klebsiella pneumoniae* and *Klebsiellaoxitoca* in Japan. The present study was conducted to find out the recent prevalence and antimicrobial susceptible patterns of both *Klebsiella pneumoniae* and *Klebsiellaoxitoca*isolates at antertiary care university hospital in the central of Japan.

MATERIALS AND METHODS

Strains and clinical data collection

A total of seven hundred ten*Klebsiella* species were obtained from various clinical specimens at Nagoya City University

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hospital from 2008 to 2010. Nagoya City University hospital is an 808-bed tertiarycare university hospital in the central region of Japan. We used medical records appended to clinical species for the analysis of clinical feature at Nagoya City University Hospital. We considered several isolates from the same region of the same patient as one isolate per one patient for the analysis in this study. All *Klebsiella* isolates were identified by standard conventional biochemical methods or the VITEK2 system (bioMérieux, Durham NC, USA). Our experimental design was approved by the ethics committee at Nagoya City University.

Antimicrobial susceptibility analysis

Klebsiella species isolates were examined for 12 antibiotic susceptibilities as follow CEZ; cefazolin, CAZ; ceftazidime, CTX; cefotaxime, CFPM; cefepime, IPM; imipenem, MEPM; meropenem, MINO; minocycline, CPFX; ciprofloxacin, LVFX; levofloxacin, ST; Trimethoprim-sulfamethoxazole, GM; gentamicin, AMK; amikacin. Minimal inhibitory concentration (MICs) were determined using broth micro dilution methodology with the VITEK2 system. Evaluation of antibacterial resistance was based on Clinical Laboratory Standard Institute (CLSI) break point (M100-S20). For the purposes of this study, isolates showing *in vitro* resistance to CAZ or CTX were classified as ESBL-producing organism (Sohn *et al.*,2011). Multidrug resistance (MDR) was defined as non-susceptibility to more than any three antimicrobial agents (Magiorakos *et al.*, 2012).

Statistical analysis of the data

We conducted the statistical analysis with the chi-squared test or Fisher's exact test when appropriate. Differences were considered significant when p was <0.05.

RESULTS

Seven hundred ten *Klebsiella* species were isolated in this study. Of them, five hundred fifty*Klebsiellapneumoniae*, and one hundred sixty *Klebsiellaoxitoca*were isolated. Next, we compared the differences of various characteristics between *Klebsiella pneumoniae* and *Klebsiellaoxitoca* in this study. Three hundred eighty-five isolates (54.2%) were from male and 325 (45.8%) were from female (Table 1). The age incidence among 0-1 years age group was 83 (11.7 %) [*Klebsiellapneumoniae*-35, *Klebsiellaoxitoca*-48] (p<0.001), among1-10 years age group, 23(3.2%) [*Klebsiella pneumoniae*-13, *Klebsiella oxitoca*-10](p=0.0145), among 11 - 40 years age group, 46(6.5 %) [*Klebsiella pneumoniae*-37, *Klebsiella oxitoca*-9], in 41-60 years it was 81 (11.4 %)[*Klebsiella pneumoniae*-71, *Klebsiella oxitoca*-10](p=0.0197), in 61-75 years age group it was 224 (31.5 %) [*Klebsiella pneumoniae*-179, *Klebsiella oxitoca*-45] and >75 years age group it was 253 (35.6 %) [*Klebsiella pneumoniae*-215, *Klebsiellaoxitoca*-38](p=0.0004) (Table 1). Four hundred seventy isolates (66.2%) were from outpatient and 240 (33.8%) were from inpatient(p=0.02) (Table 1). In our study, sputum 216(30.4%), urine 214 (30.1 %) were the source of *Klebsiella* isolates(Table 2). Although isolation of *Klebsiella pneumoniae* from sputum was significant greater than that of *Klebsiellaoxitoca*, isolation of *Klebsiellaoxitoca* from nose was significant greater than that of *Klebsiella pneumoniae* (p<0.01). Most of the *Klebsiella* species isolates were from the urology (149/21.0%) followed by paediatrics (81/11.4%), respiratory medicine (72/10.1%), intensive care unit (65/9.2%) and surgery (60/8.5%) and lowest from ophthalmology (1/0.1%), and oral surgery (1/0.1%) (Table 1).

Table 1 Demographic and clinical characteristics patterns of *Klebsiellapneumoniae* and *Klebsiellaoxitoca* infection

Gender		*Klebsiellapneumoniae*	*Klebsiellaoxitoca*	*p*value
	Female	241	84	0.0524
	Male	309	76	
Age				
	0-1	35	48	<0.001
	1-10	13	10	0.0145
	11-40	37	9	0.6181
	41-60	71	10	0.0197
	61-75	179	45	0.2896
	>75	215	38	0.0004
Hospitalization				
	Inpatient	352	118	0.0218
	Outpatient	198	42	
Department				
	ICU	51	14	0.8401
	Psychiatry	3	0	0.8074
	Ophthalmology	1	0	0.5107
	Emergency medici	25	4	0.3557
	Haematology	9	1	0.5657
	Respiratory medici	58	14	0.5079
	Gynaecology	1	1	0.9334
	Oral surgery	1	0	0.5107
	Otolaryngology	8	4	0.5792
	Cardiology	20	1	0.0866
	Paediatrics	26	55	<0.001
	Paediatric surgery	6	0	0.4031
	Surgery	49	11	0.4156
	Gastroenterology	26	3	0.1684
	Cardiac surgery	2	1	0.8074
	Neurology	15	3	0.7506
	Nephrology	14	3	0.8458
	Orthopaedics	5	0	0.5008
	General medicine	24	6	0.7342
	Endocrinology	5	0	0.5008
	Neurosurgery	29	2	0.0486
	Urology	118	31	0.5697
	Dermatology	35	4	0.0909
	Radiology	4	0	0.63
	Anaesthesiology	3	0	0.8074
	Rheumatology	12	2	0.6722
Biological sources				
	Device	19	10	0.1159
	Pharyngeal mucus	45	16	0.4701
	Pus	33	4	0.1209
	Eye discharge	1	0	0.5107
	Pleural effusion	1	0	0.5107
	Oral cavity	1	1	0.9334
	Ear discharge	4	2	0.8846
	Effusion	18	8	0.3059
	Blood	22	4	0.5157
	Bile	18	2	0.2759
	Urine	166	48	0.9648
	Skin	8	1	0.6715
	Nose	10	31	<0.001
	Ascites	16	3	0.6635
	Sputum	188	28	<0.001
	Vaginal discharge	1	1	0.9334

Although isolation of *Klebsiella pneumoniae* from neurosurgery was significant greater than that of *Klebsiellaoxitoca*, isolation of *Klebsiellaoxitoca* from paediatrics was significant greater than that of *Klebsiella pneumoniae* (p<0.01).

Table 2 Antibacterial resistant rates of *Klebsiella pneumoniae* and *Klebsiellaoxitoca* isolates

	Klebsiella pneumoniae	***Klebsiellaoxitoca***	***p*value**
Antibiotics			
CEZ	39	66	<0.001
CAZ	18	3	0.5135
CTX	29	3	0.1081
CFPM	20	3	0.3932
IPM	2	2	0.4725
MEMP	0	1	0.5107
MINO	82	42	0.0002
CPFX	18	5	0.926
LVFX	16	4	0.9057
ST	31	1	0.0134
GM	25	0	0.0124
AMK	0	0	
ESBL			
Positive	31	4	0.1599
Negative	519	156	
Multi drug resistant			
ESBL positive	9	0	1
ESBL negative	8	1	

The results of antibacterial susceptibility of *Klebsiella*species isolates to various antibiotics tested in this study are shown in Table 2. The best antibiotics with over 99% susceptibility rates were amikacin (100%), meropenem (99.9%) and imipenem (99.4%).Significant resistant were observed in minocycline (124/17.5%) and cefazolin (105/14.8%). Although resistant rate to minocycline, trimethoprim-sulfamethoxazole, and gentamicin in *Klebsiella pneumoniae* was greater than that in *Klebsiellaroxitoca*, resistant rate to cefazolin*in Klebsiellaoxitoca* was greater than that in *Klebsiella pneumoniae*(p<0.01). The numbers of ESBL isolates were thirty-five (4.9%). Furthermore, our study revealed that eighteen*Klebsiella* species isolates had multidrug resistant ability (Table 2). The nine *Klebsiella pneumoniae*had multi drug resistantactivity including ESBL.

DISCUSSION

In this study, we described the characteristics of *Klebsiella* species isolates from 2008 to 2010 at a tertiary care university hospital in the central region of Japan.

From the point of view of gender group, *Klebsiella* species were isolated more from male than female. Our study showed the male to female ratio was about 1.2 time and there was no significant differences among gender group.

Next, we clarified *Klebsiella* species with age distribution. The present study reveals the prevalence of *Klebsiella* species as seen in 0-1 years age group, it is 11.7%, decreasing to 3.2% in 1-10 years age group, increasing to 6.5% in 11-40 years age group again and to 11.4% in 41- 60 years age group, and to 31.5% in 60- 75 years age group, and finally to 35.6% in more than 75 years age group. Although young patients under 10 years frequently caused *Klebsiella* species infection, the about two-third of *Klebsiella* species were isolated from over 60 years age patients in our study. It is suggested to decrease immunity in the extremes of age groups.

With respect to hospitalized group, *Klebsiella* species were isolated more from outpatient than inpatient. Our study showed the outpatient to inpatient ratio was about 2 time and there were significant differences among hospitalization. Our result represented that *Klebsiella* species is one of important nosocomial pathogens.

In the analysis of biological sources, we found that biological sources where most patients with *Klebsiella* species were detected was sputum and urine. Furthermore, in the analysis of clinical departments, we found that department where most patients with *Klebsiella* species were detected was urology, paediatrics, and respiratory medicine. Our result showed that urinary and respiratory tract diseasewere usually popular as *Klebsiella* infectious diseases (Broberg CA, *et al*, 2014). Pyogenic liver abscesses (PLA) is serious *Klebsiella* infectious disease (Liu YC, *et al*, 1986). Our result showed that only one *Klebsiella pneumoniae* in presumably PLA was isolated by aspiration method at department of gastroenterology. But this isolates were susceptible to all twelve antibiotics. Previous study also demonstrated that ESBL production in *Klebsiella pneumoniae* was negatively correlated with invasive syndrome such as PLA (Lee CH, *et al*, 2010). Some researchers suggested that ESBL-producing *Klebsiella pneumoniae* actually have distinct bacterial characteristics (TogawaA, *et al*,2015)

Although the disease burden of *Klebsiella* species infections has increased due to widespread emergence of antibacterial resistance in many countries from1980s (Turner PJ, *et a.*, 2005), our result showed that about 75% of *Klebsiella* species were susceptible to all antibiotics tested.

Surprisingly, cefazolin showed > 80% *in vitro* effectiveness against *Klebsiella* species in our study. This result is consistent with previous East Asian study (Chen LF, *et al*, 2013).

Antibacterial susceptible analysis of *Klebsiella*species in our study revealed that minocycline wasnot first-line effective antibiotics against these bacteria because minocycline resistant rates of *Klebsiella* species were 17.4%. Especially, we found the low effective of minocycline against *Klebsiellaoxitoca* because resistant rate was about 26%.

Although carbapenem-resistant Enterobacteriaceae are particlulary problem worldwide (Kang CI, *et al*, 2013), our result showed that the carbapenems such as imipenem and meropenem were effective against *Klebsiella* species. Only 4 *Klebsiella* species had no carbapenemsusceptibility.

The overall rates of resistance to fluoroquinolone in *Klebsiella* species remained low (3.3%) in our study. Other report showed that about 97% of *Klebsiella* species had ciprofloxacin susceptibility in Japan (Yanagihara K, *et al*,2015) and was consistent with our result.

Previous studies documented that MYSTIC study, in which, overall, ESBL phenotypes among global *Klebsiella*

pneumoniae were 18.5% (Turner P, *et al*, 2005). However, ESBL producing *Klebsiella pneumonia* was very prevalent in Eastern Europe (58.7%) followed by Asian countries and southern Europe (28.2 % and 24.4%, respectively), and contrasting with the 16.7% and 12.3% in northern Europe and North America, respectively (Turner P, *et al*,2005). Our result is almost consistent with previous Asian result of ESBL producing *Klebsiella* species.

From our result, multi drug resistance rate of *Klebsiella* species was about 4.9% in Japan.

While KPC-producing organisms are rarely reported in Asian country, NDM-producing organisms are prevent among *Klebsiellapneumoniae* in India and Pakistan (Nordmann P, *et al*, 2009). NDM-1 carbapenemase-producing Enterobacteriaceae strains have been detected worldewide (Jeans SS, *et al*,2011). Henceforth we need to focus on the antimicrobial susceptible pattern in *Klebsiella* species.

CONCLUSION

Incidence of antibiotics resistant *Klebsiella* species infection is increasing, both in healthcare institutions and throughout communities, in many countries such as Asia. Continuous surveillance is essential for providing information on the trends in antibacterial resistance.

Comprehensive strategies for the control of antibacterial resistance are urgently desired in Asia including Japan.

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