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Studies on *Pasteurella multocida* isolated from pneumonic lungs of slaughter pigs

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도축돈의 폐렴병소에서 분리한 *Pasteurella multocida*에 대한 연구

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초록 : 이 실험은 도축돈의 폐에서 *Pasteurella multocida* 감염의 발생빈도를 조사하고 분리된 *Pasteurella multocida*의 항생제에 대한 약제감수성, 생화학적특성, 협막혈청형의 분류 및 독소생성능에 대해서 조사하였다. 실험재료로는 영남지방의 돼지 450두가 사용되었으며, 재료채취는 1992년 4월부터 1993년 3월 사이에 시행되었다.

*P multocida*는 도축돈의 폐 450예에서 80주가 분리되어 17.7%의 분리율을 나타내었다. 분리균 대부분의 생화학적 및 배양성상은 reference strain의 것과 일치하였으며, 분리균 80주의 협막혈청형을 동정한 결과 77주가 type A, 나머지 3주가 type D로 나타나 각각 96.3%, 3.8%의 분포비를 나타내었다. 항생제에 대한 감수성검사에서 모든 분리균주는 ampicillin, ceftiofur, cephalothin, ciprofloxacin, penicillin-G 등에 대해서는 매우 감수성이 높았으며 이들 분리균 중 일부는 sulfadimethoxine과 streptomycin에 내성을 나타내었다. 분리균 80주중 61주가 독소생성능이 있는 것으로 나타났으며, type A 77, type D 3주중 76.6%가 각각 독소생성능이 있는 것으로 나타나 협막혈청형간에 독소생성능의 뚜렷한 차이가 인정되지 않았다.

Key words: *Pasteurella multocida*, capsular serotypes, toxigenicity

Introduction

Pasteurella multocida is the causative agent of pasteurellosis in variety of animal hosts, and can develop pneumonia and atrophic rhinitis in pigs. Consisting of complicated antigenic structure, *P multocida* has 5 capsular serotypes(A, B, D, E and F) and 16 somatic serotypes[18]. The capsule which *P multocida* produces is a very important virulence factor, especially of serotype A, for it helps the organism avoid phagocytosis by alveolar macrophage[17,18]. So serotype A plays an important role in bringing about pneumonia more than serotype D does. Dermonecrotic toxin(DNT), one of the major virulence factors of *P multocida*, is central to the production of atrophic rhinitis, where only toxigenic strains of *P multocida* are involved in the disease[18,21,22]. Although single infection of *P multocida* can cause pneumonia, *P multocida*

is not a primary agent of pneumonia. But respiratory disease complicated by *P multocida* is present to a varying degree in most growing finishing pigs and the most common bacterial disease of pigs in the world[18,19]. The available evidences suggest that *P multocida* has been most frequently isolated from swine lungs and causes a lot of economic losses in swine industry[23,26]. Toxigenic strains of *P multocida* from swine lungs have been reported by a number of authors[5,8,10,17,23,24], and the importance of dermonecrotic toxin(DNT) produced by *P multocida* has been gradually increasing. But there have not been enough reports about not only toxigenicity of *P multocida* but also the incidence of *P multocida* infection in Korea[3,11,16]. So the objective of this work was to investigate the incidence of *P multocida* infection in the lung of slaughter pigs, and to examine the biochemical and cultural characteristics, antimicrobial susceptibility, capsular serotypes and toxigenicity of the isolates.

Materials and Methods

Collection of lung samples: 450 lung samples of slaughter pigs with gross lung lesions were collected from Taegu slaughter plant during the period from April 1992 to March 1993.

Macroscopic examination: All lungs were evaluated by the same person, and the gross lesions were qualitatively grouped with the special regard to uncomplicated mycoplasma pneumonia, complicated pneumonia and complicated pneumonia with pleuritis[20].

Isolation of *P multocida*: Isolation of *P multocida* was performed by inoculating the lung suspensions on sheep blood agar. The plates were incubated at 37℃ and examined after 18-24 hrs.

Biochemical and cultural characteristics: All biochemical and cultural procedures were carried out according to Cowan and MacFaddin's methods[4,12].

Antimicrobial susceptibility test: The test was performed by agar plate dilution method. Antibiotics used were of Sigma product except ceptiofur(Upjohn).

Capsular serotyping of *P multocida* isolates: *P multocida* isolates ware identified as capsular serotype A and D by methods described by Carter and Rundell[1], and Carter and Subronto.[2]

Detection of dermonecrotic toxin(DNT): The isolates were tested for toxin production by modified De Jong and Sawata's method[5,25]. *P multocida* isolate incubated in tryptic-soy broth at 37℃ for 18 hrs was sonicated twice for 20 min and centrifuged at 3000rpm for 60 min at 4℃(sonicator: Samboultrasonic Co Model:SB 150). The supernant fluid was passed through a 0.2μm membrane filter and designated as a test sample. 0.5ml of bacterium-free broth-filtrate were intraperitoneally injected into mouse(ICR from life science) weighing 15-20g. Toxigenic *P multocida* killed a mouse in 1-10 days.

Results

Isolation frequency of *P multocida* from pneumonic lungs of slaughter pigs is shown in Table 1. *P multocida* was isolated from 80(17.7%) of 450 pneumonic lungs of slaughter pigs. Isolation rate of *P multocida* from complicated pneumonia with pleuritis was higher than those of remaining two lesions. Among 80 isolates seventy seven strains(96.3%) were capsular serotype A and the remaining 3(3.9%) were capsular serotype D(Table 2). Capsular serotype D was isolated from complicated pneumonia with pleuritis, but not

Table 1. The isolation frequency of *Pasteurella multocida* from pneumonic lungs of slaughter pigs

Types of lung lesions	No of lungs examined	No of *P multocida* isolated	Percent of *P multocida* isolated
Uncomplicated mycoplasma pneumonia	32	2	6.3
Complicated pneumonia	343	57	16.6
Complicated pneumonia with pleuritis	75	21	28.0
Total	450	80	17.7

Table 2. Capsular serotypes of 80 isolates of *Pasteurella multocida* from pneumonic lungs of slaugter pigs

Sources of isolates	No of isolates	Capsular serotypes	
		Type A	Type D
Uncomplicated mycoplasma pneumonia	2	2(100)*	0(0)
Complicated pneumonia	57	57(100)	0(0)
Complicated pneumonia with pleuritis	21	18(85.7)	3(14.3)
Total	80	77(96.3)	3(3.7)

* Figures in parentheses are percentages.

Table 3. Biochemical and cultural properties of 80 isolates of *Pasteurella multocida* from pneumonic lungs of slaughter pigs

Properties	No of positive isolates	Percent of positive isolates
Catalase	80	100.0
Growth on MacConkey agar	0	0.0
Hydrogen sulfide production(lead acetate paper)	75	93.8
Indole production	73	91.3
Motility	0	0.0
Urease production	0	0.0
Nitrate reduction	80	100.0
Hemolysis	0	0.0
Methyl-Red reaction	0	0.0
Voges-Proskauer reaction	0	0.0
Gelatin liquefaction	0	0.0
Oxidase	80	100.0

Table 4. Carbohydrate fermentative properties of 80 isolates of *Pasteurella multocida* from pneumonic lungs of slaughter pigs

Fermentable substrates	No of positive isolates	Percent of positive isolates
Arabinose	27	33.8
Dulcitol	34	42.5
Galactose	80	100.0
Glucose	80	100.0
Inositol	30	37.5
Inulin	30	37.5
Lactose	42	52.5
Maltose	33	41.3
Mannitol	69	82.3
Raffinose	23	28.8
Salicin	32	40.0
Sorbitol	75	93.8
Sucrose	80	100.0
Trehalose	62	77.5
Xylose	75	93.8

Table 5. Antimicrobial susceptibility of 80 isolates of *Pasteurella multocida* from pneumonic lungs of slaughter pigs

Antimicro-	No of cultures with MIC(μg/ml or IU/ml)											
bials	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	100<
AK						1	15	53	11			
AM	67	12	1									
CE	13	42	17	5		2	1					
CF	59	14		2		4	1					
CIP	80											
CP	2	1	12	65								
EM	1	1	5	31	28	13	1					
KM					1	17	53	9				
LM							1	52	9	17	1	
OT			3	3	3	18	3	26	22	2		
PG	13	36	24		1		1	5				
SDM							3	15	39		10	13
SM					2	4	21	31	3		10	9

AK: amikacine, AM: ampicillin, CF: ceftiofur, CE: cephalothin, CIP: ciprofloxacin, CP: chloramphenicol, EM: erythromycin, KM: kanamycin, LM: lincomycin, OT: oxytetracyclin, PG: penicillin-G, SDM: sulfadimethoxine, SM: streptomycin

Table 6. Toxigenicity of 80 isolates of *Pasteurella multocida* from pneumonic lungs of slaughter pigs

Capsular type	No of isolates	No of DNT+ve	% of DNT+ve
A	77	59	77
D	3	2	67
Total	80	61	76

from other two lesions. The biochemical and cultural characteristics of *P multocida* isolates are shown in Table 3. All isolates were catalase positive, nonmotile, urease negative, nitrate negative, nonhemolysis, MR-VP negative and oxidase positive, and did not grow well on MacConkey agar. Table 4 presents carbohydrate fermentative properties of 80 *P multocida* isolates. All isolates were positive to galactose, glucose and sucrose.

Antimicrobial susceptibility of *P multocida* isolates is shown in Table 5, and most of isolates were susceptible to antibiotics used. Sixty one(76.3%) of all 80 *P multocida* isolates were dermonecrotic toxin producers (Table 6). Out of type A and type D isolates, 59(76.6%) and 2(66%) were toxigenic, respectively.

Discussion

P multocida is very important to pneumonia and atrophic rhinitis in pigs. According to latest reports, not only serotype D but also serotype A can develop atrophic rhinitis in pigs[24] and artificial single infection of *P multocida* into pigs can give rise to atrophic rhinitis and pneumonia[6,7].

In this study, isolation rate of *P multocida* from pneumonic lungs was relatively lower than those reported by other authors[3,8,9,15,17]. The isolation rate of *P multocida* from pigs they reported, according to circumstance, was very various although *P multocida* was isolated from the same pneumonic lungs. The diversity of isolation rate of *P multocida* may be in difference of isolation methods[17] and effect of antibiotics remaining in slaughter pigs. The majority of lung isolates of

P multocida were type A, and type D strains encounted on rare occasion. This result was very similar to those of other reports[3,8,9,14,17]. That type A is frenquently isolated from lungs is because the capsule, an important virulence factor, prevents alveolar macrophages from phagocyting organisms[13,17].

The majority of biochemical and cultural characteristics were identical to those of the reference strains employed. All isolates were very susceptible to ampicillin, ceftiofur, cephalothin, ciprofloxacin and penicillin-G, although some of them were resistant to sulfadimethoxine and/or streptomycin. In this work, 61 of 80 isolates were toxin producers, and out of 77 type A and 3 type D isolates, 59(76.6%) and 2(66.3%) were toxigenic. As these results, toxigenicity was comparatively higher than those of other reports[10,17], and the percent of toxigenic serotype A strains were higher than those of Pijoan et al.[17] and Iwamatsu and Sawada[10]. But toxigenicity of serotype D isolates was relatively lower than those of Pijoan et al[17], Iwamatsu and Sawada,[10] and H ϕ ie[9]. A number of authors have studied about toxigenic strains(both type A and type D) and the importance of toxigenic strains in pneumonic lungs has been increasing. In general, toxigenicity of type D is relatively higher than that of type A. However this result shows that the difference between capsular serotype A and D was not noted in dermonecrotic toxigenicity of the isolates. The difference of toxigenicity, among *P multocida* isolates from swine lungs, may be in detection methods of dermonecrotic toxin(DNT)[13,22] and severe degree of lung lesions.[8]

Summary

P multocida was isolated from 80(17.7%) of 450 pneumonic lungs of slaughter pigs. The majority of the biochemical and cultural characteristics of *P multocida* isolates were identical to those of the reference strains employed. Seventy seven strains(96.3%) among 80 isolates were capsular serotype A while the remaining 3(3.8%) were serotype D. All isolates were very susceptible to ampicillin, ceftiofur, cephalothin, ciprofloxacin and penicillin-G although some of them were resistant to sulfamethoxin and/or streptomycin. Sixty one(76.3%) of all 80 *P multocida* isolates were dermonecrotic toxin producers. Out of 77 isolates of serotype A and 3 isolates of serotype D, 59(76.6%) and 2(66.7%) were toxigenic, respectively. No difference was noted in dermonecrotic toxigenicity of the isolates in relation to capsular serotypes.

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