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O-Antigen Serotypes and Type III Secretory Toxins in Clinical Isolates of *Pseudomonas aeruginosa*

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The association of O-antigen serotypes with type III secretory toxins was analyzed in 99 clinical isolates of *Pseudomonas aeruginosa*. Isolates secreting ExoU were frequently serotyped as O11, but none were serotype O1. Most of the isolates that were nontypeable for O antigen did not secrete type III secretory toxins.

Lung infections caused by *Pseudomonas aeruginosa* are frequently associated with a high rate of mortality, particularly in immunocompromised patients (1, 4). In addition to an increase in the prevalence of drug-resistant organisms, these poor outcomes of *P. aeruginosa* pneumonia appear to be due to the development of acute lung injury and septic shock (1, 3, 4, 28). Among the various virulence factors of *P. aeruginosa*, lung injury and sepsis in infected hosts depend largely on the expression of exoenzyme S and its coregulated toxins secreted by the type III secretion system (TTSS) (10, 15, 29, 30, 34). The TTSS, which delivers toxins directly into the cytosol of cells, is utilized by most pathogenic gram-negative bacteria (11, 14).

The TTSS, including secretion, translocation, and regulation apparatuses, is encoded by the exoenzyme S regulon in *P. aeruginosa* (10, 33). However, the genes for the type III secretory toxins (TTS toxins) are distributed in various regions of the *P. aeruginosa* chromosomal DNA separate from the exoenzyme S regulon (8, 26). To date, four TTS toxins have been identified in *P. aeruginosa* (10, 33). ExoS (exoenzyme S) and ExoT (exoenzyme T), having ADP-ribosyltransferase activities, diminish macrophage motility and phagocytosis (12) and are associated with mortality in animal models (2, 20–22). ExoY possesses adenylate cyclase activity and affects cell morphology (32). ExoU, a cytotoxin, contributes to epithelial cell toxicity, lung injury, and sepsis in infected animals, but the mechanism of its action remains unknown (8, 16).

While almost all strains of *P. aeruginosa* appear to possess a set of genes for the TTSS itself (7, 13), not all strains carry genes for all of the four TTS toxins. For instance, strain PAO1 has a negative genotype for *exoU* and strain PA103 has a negative genotype for *exoS* (8, 9, 26). In addition, some chronic isolates suppress the expression of the TTSS (24). It has been reported that patients infected with *P. aeruginosa* expressing the TTSS had a sixfold higher rate of mortality and an increased incidence of bacteremia than patients infected with *P. aeruginosa* not expressing the TTSS (24). A poor prognosis for patients with ventilator-associated pneumonia due to *P. aeruginosa* is associated with strains expressing the TTSS (13). Therefore, characterizing the phenotypes of TTS toxins in *P. aeruginosa* isolates could help in the identification of virulent strains.

The lipopolysaccharide (LPS) O antigen has been used for the classification of *P. aeruginosa* isolates. There are 20 different International Antigenic Typing Scheme serotypes of *P. aeruginosa* based on differences of the B-band LPS. Our group previously obtained 108 clinical isolates of *P. aeruginosa*, and 99 of these were found to be unique clonal strains by DNA fingerprinting using enterobacterial repetitive intergenic consensus- and random amplified polymorphic DNA-PCR methods (18, 19, 24). In this study, we classified the 99 isolates by O-antigen serotypes (O serotypes), from O1 to O17, with serotype-specific monoclonal antibodies (*P. aeruginosa* serotyping kit; ERFA, Westmount, Quebec, Canada) and then determined the associations between O serotypes and TTS toxin phenotypes.

Prevalences of O serotypes and clinical association. The O serotypes of 62 of the 99 isolates (62.6%) were determined, while the remaining 37 isolates (37.4%) were nontypeable (O^-) (Table 1). O6 (14.1%) was found to be the most prevalent serotype among the typeable isolates. In addition, 13.1, 9.1, and 8.1% of the isolates were found to be serotypes O1, O11, and O4, respectively. Serotypes O12 to O17 were not found among any of the isolates. Serotype O1 isolates were more frequently isolated from patients with acute infections than from those with chronic infections (cystic fibrosis) (17.6 versus 3.2%) (Table 2). All serotype O11 isolates were from patients with acute infections (13.2% of the isolates from patients with acute infections were serotype O11 versus none of those from patients with chronic infections) (Table 2). Isolates that were nontypeable for O antigen were more frequently isolated from patients with chronic infections than from those with acute infections (67.7 versus 23.5%) (Table 2). Among the 17 isolates that were directly associated with patients' deaths (24) (Table 1), those serotyped as O4 were frequently associated with mortality (23.5% of the isolates associated with mortality were serotyped as O4, while only 4.9% of the isolates which were not associated with mortality were serotyped as O4 [Table 2]).

Associations between O serotypes and secretion of TTS tox-

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TABLE 1. LPS O-antigen serotypes in the clinical isolates of *P. aeruginosa* and association with patient mortality

O-antigen type	No. (%) of isolates from patients with:		Total no. (%) of isolates with indicated serotype[b]	No. (%) of isolates of each serotype associated with mortality
	Acute infection	Chronic infection		
O1	12 (12.1)[a]	1 (1.0)	13 (13.1)	3 (23.1)
O2	0 (0.0)	2 (2.0)	2 (2.0)	0 (0.0)
O3	3 (3.0)	1 (1.0)	4 (4.0)	0 (0.0)
O4	7 (7.1)	1 (1.0)	8 (8.1)	4 (50.0)[a]
O5	3 (3.0)	0 (0.0)	3 (3.0)	0 (0.0)
O6	11 (11.1)	3 (3.0)	14 (14.1)	4 (28.6)
O7	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)
O8	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)
O9	2 (2.0)	2 (2.0)	4 (4.0)	0 (0.0)
O10	3 (3.0)	0 (0.0)	3 (3.0)	0 (0.0)
O11	9 (9.1)[a]	0 (0.0)	9 (9.1)	3 (33.3)
O12–O17	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
O$^-$	16 (16.2)	21 (21.2)[a]	37 (37.4)	3 (9.0)
Total	68 (68.7)	31 (31.3)	99 (100)	17 (17.2)

[a] Association analyzed by Fisher's exact test in Table 2.
[b] 62 (62.6%) of the isolates were serotypes O1 to O11.

ins. The TTS toxin phenotypes of the 99 isolates classified by specific O serotypes are shown in Table 3. None of the serotype O1 strains secreted ExoU, while 16.7% of the isolates that did not secrete ExoU were serotype O1 (Table 4). Strains that secreted ExoU were frequently serotyped as O11 (33.3%) (Table 4). The isolates that secreted none of the three TTS toxins were rarely classified as O11 (2.0%) (Table 4). Most of the isolates that were nontypeable for O antigen (64.9%) did not secrete any of the three TTS toxins (Tables 3 and 4). A nontypeable serotype for O antigen was associated with a negative phenotype for ExoT, ExoU, or all three TTS toxins (Table 4).

Serotype O11 strains are common in the environment and in hospital outbreaks and have recently been shown to exhibit multidrug resistance (6, 27). Di Martino et al. reported that all of the O11 isolates of *P. aeruginosa* that they collected from patients in intensive care units were adherent and cytotoxic (5). Strain PA103 has been used for characterization of numerous virulence factors in various models of infection; it secretes ExoU and is a member of serotype O11 (8, 9, 25). According to the review by H. L. Rocchetta et al., a copy of IS*1209*, which was found in a B-band O-antigen gene cluster in serotype O5 *P. aeruginosa*, is located upstream of *exoU* in PA103 (23). Therefore, the phenotypic relationship between serotype O11 and ExoU may be based on unknown genomic mechanisms.

Lam et al. reported that 68% of strains derived from cystic fibrosis patients did not express O antigen and were nontypeable (17). *P. aeruginosa* isolates from sputa of cystic fibrosis patients produced small amounts of virtually all of the tested exoproducts, including protease, elastase, phospholipase C,

TABLE 2. Associations between LPS O-antigen serotypes and patients' diseases

Serotype[a]	No. of isolates from patients (% of total in subgroup) with:		Relative risk (95% confidence interval)
	Acute infection (n = 68)	Chronic infection (n = 31)	
O1	12 (17.6)[b]	1 (3.2)	1.4 (1.1–1.8)
O11	9 (13.2)[c]	0 (0.0)	1.5 (1.3–1.8)
O$^-$	16 (23.5)[d]	21 (67.7)	3.5 (1.9–6.6)

[a] Serotype O4 was found in 4 (23.5%) of the 17 isolates from patients who died and in 4 (4.9%) of the 82 isolates from patients who survived (P = 0.03; relative risk, 3.5 [95% confidence interval, 1.5 to 8.2]).
[b] Significantly different from chronic infection group, P = 0.04 (Fisher's exact test).
[c] Significantly different from chronic infection group, P = 0.03 (Fisher's exact test).
[d] Significantly different from chronic infection group, $P < 0.0001$ (Fisher's exact test).

TABLE 3. LPS O-antigen serotypes and TTS toxin phenotypes in the clinical isolates of *P. aeruginosa*

O-antigen type (no. of isolates)	No. (%) of isolates with phenotype:			
	ExoS$^+$	ExoT$^+$	ExoU$^+$	ExoS$^-$T$^-$U$^-$
O1 (13)	7 (53.8)	4 (30.8)	0 (0.0)[a]	6 (46.2)
O2 (2)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)
O3 (4)	2 (50.0)	2 (50.0)	0 (0.0)	2 (50.0)
O4 (8)	2 (25.0)	4 (50.0)	3 (37.5)	3 (37.5)
O5 (3)	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)
O6 (14)	6 (42.0)	6 (42.9)	2 (14.3)	6 (42.9)
O7 (1)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)
O8 (1)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)
O9 (4)	1 (25.0)	2 (50.0)	1 (25.0)	2 (50.0)
O10 (3)	0 (0.0)	2 (66.7)	2 (66.7)	1 (33.3)
O11 (9)	1 (11.1)	5 (55.6)	7 (77.8)[a]	1 (11.1)[a]
O12–O17 (0)	—[b]	—	—	—
O$^-$ (37)	9 (24.3)	8 (21.6)[a]	4 (10.8)[a]	24 (64.9)[a]
Total	30 (30.3)	35 (35.4)	21 (21.2)	49 (49.5)

[a] Association analyzed by Fisher's exact test in Table 4.
[b] —, not found.

TABLE 4. Associations between O-antigen serotypes and TTS toxin phenotypes

Serotype	Toxin phenotype	No. of isolates/total no. with indicated phenotype (%)
O1	ExoU$^-$	13/78 (16.7)[a]
	ExoU$^+$	0/21 (0.0)
O11	ExoU$^+$	7/21 (33.3)[b]
	ExoU$^-$	2/78 (2.6)
	ExoS$^+$, T$^+$, or U$^+$	8/50 (16.0)[c]
	ExoS$^-$T$^-$U$^-$	1/49 (2.0)
O$^-$	ExoT$^-$	29/64 (43.5)[d]
	ExoT$^+$	8/35 (22.9)
	ExoU$^-$	33/78 (42.3)[e]
	ExoU$^+$	4/21 (19.0)
	ExoS$^-$T$^-$U$^-$	24/49 (49.0)[f]
	ExoS$^+$, T$^+$, or U$^+$	13/50 (26.0)

[a] For comparison of prevalences between ExoU$^-$ and ExoU$^+$, P = 0.04 by Fisher's exact test (relative risk, 1.3 [95% confidence interval, 1.2 to 1.5]).
[b] For comparison of prevalences between ExoU$^+$ and ExoU$^-$, P = 0.0002 by Fisher's exact test (relative risk, 5.0 [95% confidence interval, 2.8 to 9.1]).
[c] For comparison of prevalences between ExoS$^+$, T$^+$, or U$^+$ and ExoS$^-$T$^-$U$^-$, P = 0.02 by Fisher's exact test (relative risk, 1.9 [95% confidence interval, 1.4 to 2.6]).
[d] For comparison of prevalences between ExoT$^-$ and ExoT$^+$, P = 0.02 by Fisher's exact test (relative risk, 1.4 [95% confidence interval, 1.1 to 1.8]).
[e] For comparison of prevalences between ExoU$^-$ and ExoU$^+$, P = 0.04 by Fisher's exact test (relative risk, 1.2 [95% confidence interval, 1.0 to 1.5]).
[f] For comparison of prevalences between ExoS$^-$T$^-$U$^-$ and ExoS$^+$, T$^+$, or U$^+$, P = 0.02 by Fisher's exact test (relative risk, 1.6 [95% confidence interval, 1.1 to 2.4]).

exotoxin A, and exoenzyme S, produced in vitro, especially compared with the amounts produced by sputum isolates of *P. aeruginosa* from patients with acute lung infections (31). Suppression of the TTSS and O antigen may be a part of a global suppression of all of the toxic exoproducts in the isolates from patients with chronic infections.

In conclusion, we found that *P. aeruginosa* isolates secreting ExoU were frequently serotyped as O11 and that none of these isolates were serotype O1. Most isolates that were nontypeable for O antigen did not secrete TTS toxins. The monitoring of O-antigen serotypes and TTS toxin phenotypes has epidemiological significance in the identification of virulent *P. aeruginosa* isolates, especially in high-risk patients, such as the artificially ventilated and the immunocompromised. A further genomic analysis of *P. aeruginosa* is required to clarify the mechanism of the correlations between *exoU* and O-antigen types.

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