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A longitudinal study of *Staphylococcus hyicus* colonization of vagina of gilts and transmission to piglets

H. C. WEGENER[1]* AND E. W. SKOV-JENSEN[2]

[1] *National Veterinary Laboratory, Department of Microbiology, Copenhagen, Denmark*

[2] *Staby-Ulfborg-Tim Veterinarians, Ulfborg, Denmark*

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SUMMARY

High *Staphylococcus hyicus* colonization rates were found in vaginal samples of healthy breeding sows and in skin samples of their offspring. Twenty-two different phage types were identified among the 720 isolates of *S. hyicus* examined. Two to 13 different phage types were isolated per herd. Phage typing, as well as characterization of about 10% of the isolates by plasmid profiles and antibiogram patterns, showed that, several different clones of *S. hyicus* could be present simultaneously in vagina of gilts and also on skin of piglets. Generally isolates from the vagina of one animal were identical as regards to phage types, plasmid profiles, and antibiogram patterns during the entire investigation period. Isolates from the skin of piglets were of the same type as their mothers, indicating that vertical transmission had taken place. *S. hyicus* strains isolated from the skin of piglets within 24 h after birth were identical to strains isolated 3 weeks after birth from the same litter, indicating that the vaginal strains became part of a stable skin flora.

INTRODUCTION

Staphylococcus hyicus is the causative agent of exudative epidermitis (EE), a generalized infection of the skin of sucklings and weaned pigs. The infection can occur in an acute, peracute or localized form [1]. Morbidity and mortality in infected herds can reach 90% [2]. *S. hyicus* has, furthermore, been described as the cause of polyarthritis [3], and abortion in pigs [4]. The disease can be treated with antibiotics, and prevention may be attempted by vaccination of sows with autogenous vaccine [2]. The strains of *S. hyicus* can be divided into virulent and avirulent strains with regard to their ability to produce EE in experimental piglets [5, 6]. *S. hyicus* is often isolated from skin of healthy pigs and piglets [7]. Both virulent and avirulent strains may be isolated simultaneously from diseased piglets [8].

The epidemiology of *S. hyicus* in pig herds is not very well known. It is generally presumed that the disease is introduced to 'clean' heads with infected carriers and that outbreaks first occur in litters with contact to carrier sows [9]. Outbreaks

* Address for reprints/correspondence: H. C. Wegener, National Veterinary Laboratory, 27 Bülowsvej, DK-1790 Copenhagen, Denmark.

often occur within a week after farrowing, or shortly after transfer of the piglets to the weaning section [10]. The offspring of non-immune gilts are believed to be especially susceptible in a herd where the disease is enzootic, but the disease may spread to all litters in the herd [9, 10]. Predisposing factors for the disease may be early weaning, bites and bruises, or parasitic skin infections [2, 10].

In the present investigation the prevalence of *S. hyicus* in vagina of gilts from four herds were studied at predetermined intervals from introduction of the prepubertal gilts into the herds, through the farrowing period and on the skin of the offspring, until 3 weeks after birth. The transmission of *S. hyicus* strains from the vagina or gilts to their offspring was studied by typing of all isolates by a phage-typing system recently developed by Wegener [8]. A number of strains were further characterized by determination of plasmid profiles and antibiogram patterns. These strains originated from gilts, and their offspring, where isolation of *S. hyicus* were possible during the entire investigation period. Plasmid profiles and antibiogram patterns were compared to the phage-typing results.

MATERIALS AND METHODS

Description of pig herds

Four healthy breeding herds with no prehistory of exudative epidermitis were chosen for the investigation. The herds are presented schematically in Table 1.

Sampling of vaginal and cutaneous microflora

Swab samples were collected from vagina of 38 prepubertal gilts in 4 different herds within 24 h after their admission to the herd, and again immediately before breeding, immediately after breeding, within 1–4 days before farrowing and within 24 h after farrowing (Table 2). The gilts purchased into the investigated herds came from different suppliers. Swab samples were collected by rigorous rubbing of the surface of the vaginal mucosa with a sterile cotton swab inserted approximately 10 cm into vagina. Contact with the surface skin was carefully avoided upon introduction and withdrawal of the swab. The swab tip was then cut off with a sterile scissor into a plastic tube containing 1 ml sterile saline. The tube was capped and mixed vigorously before transfer to the laboratory. Samples of the cutaneous microflora were collected from 3 to 5 piglets from each litter of the investigated gilts within 24 h after farrowing, and again 3 weeks after farrowing. Skin samples were collected by rubbing an approximately 10 cm^2 area behind the right ear with a sterile cotton swab previously moistened with sterile saline. The swab was further processed as described above.

Identification of S. hyicus *strains*

The samples arrived in the laboratory within 24 h after being collected in the herds. Three selective indicative agar plates [11] were immediately inoculated with 100 μl of: (*a*) undiluted sample, (*b*) 10-fold diluted sample, and (*c*) 100-fold diluted sample, respectively. If fewer than five *S. hyicus*-like colonies were seen on the (*a*) plates, the remaining sample (approximately 900 μl) was inoculated on a single plate. *S. hyicus* strains were identified as follows: clear white colonies surrounded by a white halo of precipitated lipid on selective indicative agar, which

Table 1. *Description of the four breeding herds investigated*

Herd No.	No. of sows	Breeding practice NS	Breeding practice AI	Fattening pigs	Health declaration*
I	115	+	−	450	Conventional
II	85	−	+	No	Conventional
III	87	+	−	No	SKD
IV	68	+	−	360	Conventional

* Conventional, the herd has no health certification. SKD, the herd is free from *Mycoplasma hyopneumonia* and Aujeszky's disease.

appeared non-pigmented and non-haemolytic on bovine blood agar plates (Columbia agar base, CM 331, Oxoid, supplemented with 5% sterile bovine blood), and which produced positive reactions for catalase, hyaluronidase [12], heat stable nuclease [13], and were negative for oxidase [14] and clumping factor [15], and which were resistant to bacitracin, and sensitive to furazolidone and novobiocin [16]. Sensitivity to diagnostic antibiotics was determined as described below using Neo-Sensitabs containing furazolidone (50 μg), novobiocin (5 μg), and bacitracin (0·4 U) respectively (Rosco Diagnostica, Taastrup, Denmark). Five colonies were selected at random from each sample. The strains were stored in pure culture at −80 °C in tryptic digest broth (TDB, Difco, 1829-17-8) supplemented with 10% glycerol.

Phage typing of S. hyicus *strains*

Phage typing, and interpretation of the phage typing results were performed according to previous description [8]. In brief, a phage typing system consisting of 23 different lytic phages was used. An overnight broth culture of the strain to be studied was diluted 1 : 10 before being flooded onto the surface of a TSA-C plate (tryptic soy agar, Difco, 0369-01-4 supplemented with 0·2% $CaCl_2$, $2H_2O$), which previously had been allowed to dry for 30 min at 37 °C. Five μl drops of the different phage suspensions at RTD (Routine Test Dilution) were spotted on the surface of the dry inoculated TSA-C plate by a Lidwell phage typing machine. Plates were incubated at 37 °C for 20 h. The degree of lysis produced by a given phage was recorded. Each phage was denominated a letter, and the letters of the phages producing more than 50 plaques with a given strain designated the strains phage type, for instance A/B/C. Two 'strong reaction differences' were used as differentiation criterion (a strong reaction difference being when a phage produces more than 50 plaques in one strain and no plaques in another). In the present investigation all strains were typed with the same phage-batch in order to avoid batch to batch variations.

Screening for plasmids

Plasmid DNA was prepared by a modification of the method described by Holmes and Quigley [17]. In brief, half a loopful of bacteria harvested from the surface of a blood agar plate was suspended in 350 μl STET-buffer (8% sucrose, 10 mM Tris, 50 mM-EDTA and 0·5% Triton X-100, adjusted to pH 8·0), and lysozyme (Sigma) and lysostaphin (Sigma) was added to the buffer to a final concentration of 10 and 70 μg/ml, respectively.

The mixture was incubated at 37 °C until the lysate had cleared (approximately 15–30 min). The lysate was heated in boiling water for 40 s. Chromosomal DNA and proteins were pelleted by centrifugation, and removed with an inoculation needle. Proteins were extracted with an equal volume of chloroform:isoamylalcohol (24:1), and the plasmid-DNA was precipitated by addition of equal volumes of isopropanol and NH_4Ac (150 mM/l), pelleted by centrifugation, and resuspended in 10 μl TE-buffer (10 mM/ml Tris, 1 mM/ml EDTA). Ten μl of a buffer containing 30% w/v glycerol, 0·1% bromophenol blue, 2 mM-EDTA, and 0·1 mg/ml RNase was added to the sample before electrophoresis in 0·8% agarose gels. Two plasmid preparations from each strain were examined. The plasmids from *Escherichia coli* V517 [18], and *E. coli* 39R861 [19] served as molecular weight markers.

Antibiograms

Sensitivity to antibiotics was measured by a standard tablet diffusion method on Danish blood agar plates, with the following composition: 0·35% dextrose, 0·25% hydrolysed casein Sheffield B, 0·1% starch, 1·0% beef broth, 0·33% KCl, 0·8% $Na_2HPO_4.12H_2O$, 0·006% $MgSO_4.7H_2O$, 0·08% $CaCl_2.2H_2O$, 0·001% anionic detergent, 0·04% vitamin mixture, 1·1–1·3% agar, and 5% defibrinated horse blood [20]. The tablets contained the following diffusible amount of antibiotics: 100 μg streptomycin, 80 μg tetracycline, 5·2 μg trimethoprim + 240 μg sulfamethoxazole, 5 μg penicillin, 19 μg lincomycin, 29 μg methicilin, 78 μg erythromycin, 60 μg chloramphenicol, 40 μg gentamicin, 30 μg oleandomycin, 100 μg kanamycin, 400 μg fusidic acid, respectively (Rosco Diagnostica, Taastrup, Denmark). The test was performed and interpreted according to the manufacturer's guidelines for sensitivity testing on Danish blood agar [21].

RESULTS

S. hyicus was isolated from vagina of 92% of the 38 6-month-old gilts at the time of their admission to the herds. The isolation rate ranged from 80 to 100% in the investigated herds (Table 2). Fifty percent of the vaginal samples of the same gilts were positive within 24 h before breeding, and 51% of the gilts within 24 h after breeding, the isolation rates ranging from 40 to 55% and 20 to 86% respectively (Table 2). Thirty-two percent (22–50%) of the gilts examined within 4 days before farrowing yielded *S. hyicus*. Vaginal swabs from 12 sows from 3 herds examined 24 h after farrowing were positive for *S. hyicus* in 54% of the samples (Table 2).

Five or more colonies of *S. hyicus* could be isolated from 57% of the vaginal samples. The remaining 43% of the samples yielded fewer than five colonies, indicating that the vaginas were only weakly colonized.

Skin swabs sampled within 24 h after farrowing from 154 piglets in litters from the remaining 34 gilts (4 had been culled) were positive for *S. hyicus* in 61% of the samples, ranging from 46–100% in the 4 herds (Table 2).

S. hyicus could be isolated from 74% (33–100%) of the same piglets 3 weeks after farrowing (Table 2). The carrier state of the parental gilt prior to farrowing was compared to colonization of the corresponding litter after farrowing, in 31 cases.

Table 2. *Isolation of* S. hyicus *strains from vaginae of groups of gilts at different periods and from the skin of their offspring, in each of four different pig herds*

	Herd I		Herd II		Herd III		Herd IV		All herds	
	Animals studied (*n*)	Isolation rate[a] (%)	Animals studied (*n*)	Isolation rate (%)	Animals studied (*n*)	Isolation rate (%)	Animals studied (*n*)	Isolation rate (%)	Animals studied (*n*)	Isolation rate (%)
Gilt at admission[c]	14	93	5	80	10	90	9	100	38	92
Gilt before breeding[d]	n.e.[b]	n.e.	5	40	10	50	9	55	24	50
Gilt after breeding[e]	14	86	5	20	9	22	9	44	37	51
Gilt before farrowing[f]	13	31	n.e.	n.e.	9	22	8	50	30	32
Sow after farrowing[g]	n.e.	n.e.	2	50	9	44	1	100	12	54
Piglet < 24 h[h]	63	46	12	67	44	78	35	100	154	61
Piglet 21 days[i]	63	62	12	33	42	76	43	100	160	74

[a] Percentage of animals from which *S. hyicus* could be isolated. [b] Not examined. Swab samples were collected from: [c] vaginae within 48 h after admission to the herd; [d] vaginae within 48 h before breeding; [e] vaginae within 48 h after breeding; [f] vaginae within approx. 48 h before farrowing; [g] vaginae within 24 h after farrowing; [h] skin within 24 h after farrowing; [i] skin 21 days after farrowing.

Nine of the 10 litters from which *S. hyicus* was not isolated, originated from non-carrier gilts. Twelve out of the 21 colonized litters originated from gilts which were negative within 48 h before farrowing, whereas the remaining 9 colonized litters originated from gilts from which *S. hyicus* was isolated shortly before farrowing. *S. hyicus* had been recovered earlier from all non-carrier gilts producing colonized litters, and from all non-carrier gilts producing *S. hyicus* free litters. Out of 23 litters which were colonized after farrowing, 19 were still colonized 3 weeks later. Six of the 11 litters where *S. hyicus* could not be isolated after farrowing were colonized 3 weeks later.

During the study a total of 720 isolates of *S. hyicus* were recovered from 455 samples, and 698 (97%) of the 720 isolates were typable by phages, dividing them into 22 distinct phage patterns (Table 3). The diversity of phage types found in the 4 herds ranged from 2 to 13 phage types per herd (Table 3). Strains belonging to phage-type no. 1 (pt1) could be isolated from all herds studied, 4 phage types were detected in 2 herds (pt3, pt4, pt6, pt7), and the remaining 17 phage types were identified in 1 herd only (Table 3). Phage type 1 was the dominating phage type in all herds studied, with a prevalence of 33–96% in the four herds studied (Table 3).

Plasmid profiles and antibiogram patterns were determined for strains from a number of animals and their offspring in order to further investigate the clonal relationship of the strains indicated by the strains phage types. Ten different plasmid profiles and five different antibiogram patterns were found among 80 strains from herd I studied. This series of strains consisted of nine different phage types (Table 4). The distribution of phage types among different plasmid profiles and antibiogram patterns, is also shown in Table 4. By use of all typing methods in a hierachichal system 40 different types were found among the 80 examined strains. Strains with identical phage types, collected from the same animal at different times, showed identical plasmid profiles and antibiogram patterns in 82% of the investigated cases, and strains showing different phage types, collected from the same animal, had different plasmid profiles and antibiogram patterns in 68% of 37 cases (χ^2, $P < 0{\cdot}001$).

One to four different phage types could be isolated simultaneously from vaginal swabs. An average of 1·6 different phage types were identified per sample per gilt. Skin swabs collected from 3 to 5 piglets from each of the 34 litters contained 1–6 different phage types per litter. In 23 (77%) of the 30 gilts studied, strains of identical phage types were isolated from the same gilt during the entire investigation period (approximately 5 months).

Appearance of strains with new phage types, which had previously not been present in vaginal swabs from the investigated gilts within the herd, were observed in three herds (Table 5). The plasmid profiles and antibiogram patterns of all the strains belonging to these phage types differed from those previously isolated from the same animals.

S. hyicus strains of the same phage types as previously recovered from the vagina of individual gilts were isolated from skin of their offspring in 20 (87%) of the 23 litters where *S. hyicus* could be isolated within 24 h after farrowing (Table 5). In 10 of the 11 cases studied, strains of the same phage type isolated from the litter and the mother gilt had identical plasmid profiles and antibiogram patterns.

Table 3. *Phage types of* S. hyicus *strains isolated from vaginae of gilts and skin of piglets originating from four pig herds*

Phage-type number*	Phage pattern	Prevalence of phage type in herd no. I	II	III	IV
1	D/H/I/M/N/Q/R/S/T†	51 (33%)	14 (44%)	214 (96%)	157 (52%)
2	A/B/C/H/M/N/Q/R/S/T/W	—	—	—	38 (12%)
3	E/F/G/I/J/K/L/O/P/Q/U/V/Y	—	—	2 (0·9%)	44 (14%)
4	D/E/F/G/H/I/J/K/L/M/N/O/P/Q/R/S/T/U/V/Y	47 (31%)	—	—	43 (14%)
5	E/J/P	—	—	—	3 (1·0%)
6	H/J	3 (2%)	—	—	4 (1·3%)
7	Q	3 (2%)	—	—	4 (1·3%)
8	D	—	—	—	2 (0·6%)
9	D/H/S/T	—	—	—	10 (3·2%)
10	D/F/G/H/M/N/R/S/T/X/Y	—	—	—	1 (0·3%)
11	D/H/M/N/O	—	—	—	1 (0·3%)
13	A/B/C	—	—	—	1 (0·3%)
14	A/D/F/H/K/M/P/R/S/T/U/V/W/X/Y/Z	—	10 (29%)	—	—
15	D/G/I/J/M/O/P/Q/S/U/Y	—	5 (9%)	—	—
16	H/I/K/M/N/O/P/Q/T/U/X/Y	7 (5%)	—	—	—
17	I/M/N/P/Q/T	5 (3%)	—	—	—
18	D/I/M/N/O/Q/R/S/W/X	8 (5%)	—	—	—
19	I/N/W	9 (6%)	—	—	—
20	D/H/M/N/Q/R/S/T/U/W	—	—	—	1 (0·3%)
21	N/X	11 (7%)	—	—	—
22	E/I/N/O/S	1 (0·7%)	—	—	—
NT	Not typable	8 (5%)	5 (15%)	7 (3%)	1 (0·3%)
Strains investigated		153	34	223	310

* Number used throughout this study as designation for specific phage patterns for example phage pattern E/J/P = phage type 5 = pt5.

† Phages were denominated a letter, and the strains phage type designated the letters of phages producing more than 50 plaques with a given strain.

Table 4. *Distribution of* S. hyicus *phage types among different plasmid profiles and antibiogram patterns isolated from pig herd No. I*

Plasmid profile (kb)	Number of strains	Antibiogram pattern*				
		S	ML^r	Tet^r, ML^r	Pen^r, Strep^r Tet^r, ML^r	Olea^i
No plasmids	9	4(7)†, 6(1), NT(1)				
2·45	34	1(11), NT(3), 19(1) 21(1), 22(1)	1(3), 4(7) 19(1), 21(2)	4(1)		7(2), 21(1)
2·45+3·55	8	1(3), 18(1), 19(1), 21(1)	1(2)			
2·45+3·85	6		1(1), 4(1), 16(1) 17(1), 18(2)			
2·45+4·05	12	1(1)	1(5), 16(1), 18(1) 21(1), NT(1)	4(1)	NT(1)	
2·45+4·55	2		4(2)			
2·45+3·55+ 40+50	2		4(2)			
2·45+50	3		1(2), 4(1)			
3·55	3	4(2)	4(1)			
4·05	1		4(1)			

* ^{r}, resistant to: ML, macrolides/lincosamides; Tet, tetracycline; Pen, penicillin; Strep, streptomycin; Olea, oleandomycin. S, sensitive to all antibiotics tested.

† Phage type (number of strains with the particular phage type).

Table 5. Staphylococcus hyicus *phage types isolated from vaginae of a group of gilts and skin of their offspring in each of four Danish pig herds*

	Herd I		Herd II		Herd III		Herd IV	
	Isolates studied (*n*)	Phage types[a]	Isolates studied (*n*)	Phage types	Isolates studied (*n*)	Phage types	Isolates studied (*n*)	Phage types
Gilt at admission[c]	46	1, 4, 6, 16, 17, 18, NT	17	1, 14, 15	45	1	44	1, 2, 3 4, 20
Gilt before breeding[d]	n.e.[b]	n.e.	5	1	17	1, 3, NT	23	1, 2, 3, 4, 5
Gilt after breeding[e]	34	1, 4, 16, 18, 19, 21, 22	1	1	6	1	19	1, 2, 3, 4, 7
Gilt before farrowing[f]	15	1, 4, 18, 19	n.e.	n.e.	10	1	20	1, 3, 5, 6, 7
Sow after farrowing[g]	n.e.	n.e.	4	1, 14	16	1	5	1
Piglet < 24 h[h]	23	1, 4, 7, 19	6	14, NT	62	1, NT	106	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, NT
Piglet 21 days[i]	35	1, 4, 18 21, NT	1	1	67	1, NT	93	1, 2, 3, 4, 9, 13

[a] Each distinct phage type were designated a number. The corresponding phage patterns are shown in Table 3. [b] Not examined. Swab samples were collected from: [c] vagina within 48 h after admission to the herd; [d] vagina within 48 h before breeding; [e] vagina within 48 h after breeding; [f] vagina within approx. 48 h before farrowing; [g] vagina within 24 h after farrowing; [h] skin within 24 h after farrowing; [i] skin 21 days after farrowing.

In 64% of the investigated cases, phage types present in the vagina of the gilt upon admission to the herds could also be isolated from the skin of their newborn piglets (Table 5). In all of the seven cases studied, these strains with identical phage types also showed identical plasmid profiles and antibiogram patterns.

One or more of the phage types previously detected in vagina of the mother gilt could be recovered from 19 (76%) of the 25 litters where *S. hyicus* were found 3 weeks after farrowing. In 71% of the litters these phage types were the same as those found in the gilt upon admission to the herd (Table 5). Phage types not observed in vaginae of the gilts could be recovered from skin of piglets within 24 h after farrowing in three herds. One phage type each in a single litter, in herds I and II, and 5 new phage types, 1 phage type in each of 3 litters and 2 phage types in 1 litter in herd IV (Table 5). A phage type which had not been present in the litters previously was isolated from a single litter in herd IV 3 weeks after farrowing. In the same herd, only 1 of the 5 new phage types that had been present in the skin swabs immediately after farrowing could be detected 3 weeks after farrowing (Table 5).

DISCUSSION

The isolation of *S. hyicus* from the genital tract of pigs was previously described by Amtsberg [22], who isolated *S. hyicus* from the vagina of seven sows from herds with outbreak of EE. In the present study *S. hyicus* was isolated from vagina of 35 out of 38 healthy prepubertal gilts, indicating that vaginal carriage by purchased gilts may be a potential source of infection.

There was a decline in the isolation rate of *S. hyicus* in vaginal samples collected from the gilts at the time of admission as compared to the isolation rate in samples collected at the time of breeding. This rate was even more reduced when samples were collected shortly before farrowing. This observation might reflect changes in the microenvironment in the vagina.

S. hyicus were isolated from the skin of approximately two thirds of all litters studied at birth, and from a slightly higher number of the same litters 3 weeks later. No piglets developed EE in spite of the high colonization rate of the litters. This may suggest that either the strains were avirulent, or immunity of the piglets plays a role for prevention of outbreak of the disease. More than half of the colonized litters originated from gilt which were negative in vaginal samples prior to farrowing, suggesting that other sources than vaginal mucosa might have been the source of the colonizing strains.

Isolation of *S. hyicus* strains with the same phage types, plasmid profiles and antibiogram patterns from vagina of individual gilts during the entire investigation period and on the corresponding offspring, indicates that strains carried by the gilt at the time of purchase persist in the vagina until farrowing, and that these strains can be transmitted from vagina of gilts to piglets during farrowing. Strains of the same types as the vaginal strains of the mother gilt constituted the majority of *S. hyicus* strains isolated from litters 3 weeks after birth, showing indirectly that these strains had developed into a stable skin flora.

The indigenous flora of the herds was not studied in the present investigation, and therefore its role as a source of infection cannot be evaluated sufficiently. Nevertheless, in cases where new phage types appeared in an animal, these strains

also had different plasmid profiles and/or antibiogram patterns from that of the strains previously detected in the same animal, and were thus an indication of these strains being introduced from external sources, rather than residental strains which had changed phage type. Phage types seemed in general to be stable markers during the approximately 5 month investigation period.

In conclusion, *S. hyicus* was frequently isolated from vagina of prepubertal gilts, and although the prevalence of *S. hyicus* in vagina was decreased at the time of farrowing, the majority of litters from the same gilts were colonized with *S. hyicus* less than 24 h after farrowing. The phage types, plasmid profiles and antibiograms of the isolated strains indicated that *S. hyicus* strains could be transferred from the vagina of the gilt to the piglet at birth, and that strains carried in the vagina by the gilt at the time of admission to the herd made up the majority of strains isolated from the newborn offspring. The maternal *S. hyicus* strains persisted on the skin of the offspring the first 3 weeks of the piglets life – the critical period with regard to outbreaks of exudative epidermitis.

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