News hour

VWHA Christmas Webinar

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Explor Anim Med Res,
Vol.10, Issue - 1, 2020, p. 42-49

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

ANTIBIOTIC SUSCEPTIBILITIES OF BIOFILM PRODUCING BACTERIA ISOLATED FROM HORSE WOUNDS

Nur Adilah Ahmad Nadzir[1], Zunita Zakaria[1], Noraniza Mohd Adzahan[2*], Abubakar Musa Mayaki[3,4]

Received 14 May 2020, revised 01 June 2020

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Rationale

- To demonstrate biofilm forming bacteria in equine wounds + skin
- To test antibiotic susceptibilities of biofilm producing equine wound and skin isolates

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Methods

- Wound and intact skin swabs from 30 horses (University of Malaysia)
- Standard aerobic culture
- Identification on cellular morphology, gram staining and biochemical tests

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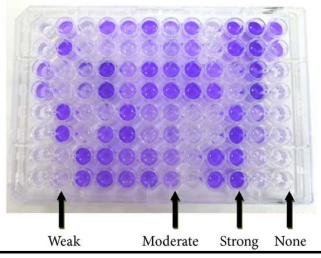
- Crystal violet microtiter plate assay to test for biofilm forming ability
  - Mild, moderate or strong biofilm former



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- Crystal violet microtiter plate assay to test for biofilm forming ability
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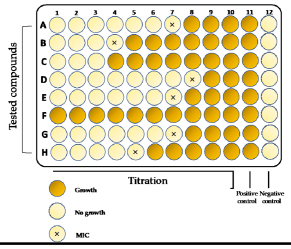
- Antimicrobial susceptibility tests
  - Ciprofloxacin, gentamycin and tetracyclin
  - Minimum inhibitory concentration (MIC) assay
  - Minimum biofilm eradication concentration (MBEC) assay



Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Results and discussion

Table1. Number and percentages of bacteria isolates from equine wound and intact skin swab samples.

	Bacteria	Wound samples		Intact Skin samples		
		Acute	Chronic	% isolates	No. of isolates	% isolates
Gram-negative	*Escherichia coli*	5	12	26.2	5	8.8
	Enterobacter spp.	8	5	20.0	12	21.1
	Acinetobacter spp.	1	2	4.6	7	12.3
	Aeromonas spp.	1	1	3.1	1	1.8
	Providencia spp.	1	1	3.1	2	3.5
	Klebsiella spp.	1	1	3.1	2	3.5
	Pseudomonas spp.	0	2	3.1	2	3.5
	Citrobacter spp.	0	1	1.5	2	3.5
	Stenotrophomonas spp.	0	0	0	2	3.5
	Serratia spp.	1	0	1.5	0	0
	Chromobacterium spp.	0	1	1.5	0	0
	Yersinia spp.	0	1	1.5	0	0
	Vibrio spp.	1	0	1.5	0	0
Gram-positive	*Staphylococcus* spp.	6	5	16.9	14	24.6
	Streptococcus spp.	0	4	6.2	2	3.5
	Corynebacterium spp.	2	0	3.1	3	5.2
	Bacillus spp.	2	0	3.1	3	5.2
TOTAL		29	36	100	57	100

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Results and discussion

Table 2. Biofilm ability of bacterial isolates from equine wounds in microtiter plate assay.

Organism	No of Isolates	Number (%) isolate from acute wound				No of Isolates	Number (%) isolate from chronic wound			
		Strong	Moderate	Weak	Non		Strong	Moderate	Weak	Non
Escherichia coli	5	0	0	3 (15.0)	2 (10.0)	12	1(3.6)	1 (3.6)	7 (25.0)	3 (10.7)
Enterobacter sp.	8	0	0	6 (30.0)	2 (10.0)	5	3 (10.7)	1 (3.6)	1 (3.6)	0
Acinetobacter sp.	1	0	0	1 (5.0)	0	2	0	0	2 (7.1)	0
Staphylococcus sp.	6	0	0	6 (30.0)	0	5	0	1 (3.6)	4 (14.3)	0
Streptococcus sp.	0	0	0	0	0	4	0	0	4 (14.3)	0
TOTAL	20	0	0	15 (80.0)	4 (20.0)	28	4 (14.3)	3 (10.8)	18 (64.3)	3 (10.7)

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Results and discussion

- MIC and MBEC of 13 isolates of *E. coli*, *Enterobacter* sp., *Staphylococcus* sp. and *Streptococcus* sp.

Table 3. The comparison between MIC and MBEC of antibiotics tested on the biofilm forming isolates from equine chronic wound.

Antibiotics (µg/mL)	Gram-negative isolates		Gram-positive isolates	
	MIC	MBEC	MIC	MBEC
Ciprofloxacin	0.27 ± 0.04	54.00 ± 12.44*	0.40 ± 0.06	25.60 ± 10.55*
Gentamycin	0.88 ± 0.18	576.0 ± 141.0*	1.30 ± 0.30	332.80 ± 76.80*
Tetracycline	2.25 ± 0.41	736.0 ± 112.8*	0.90 ± 0.29	268.80 ± 71.27*

MIC: Minimum Inhibitory Concentration; MBEC: Minimum Biofilm Eradication Concentration; Values are expressed as mean ± SEM. *Values with significantly different between MIC and MBEC

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Criticism

- Identification method

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

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- Antibiotic choice (ciprofloxacin, gentamycin and tetracyclin)

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

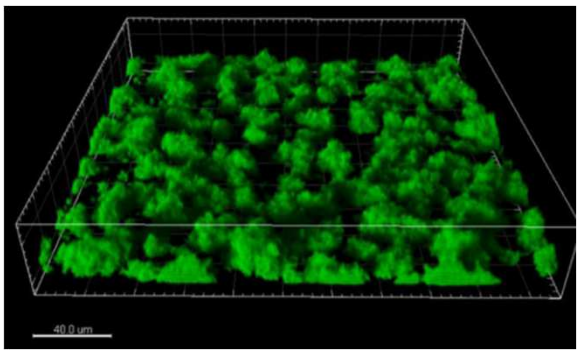
Criticism

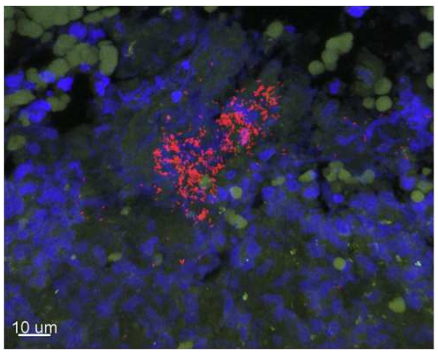
- Identification method
- Antibiotic choice
- No correlation bewteen biofilm forming abilities *in vitro* and *in vivo*

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Criticism

- *In vitro* and *in vivo* biofilms are very different, so can't be compared
  - Size and composition





Kragh *et al.* 2016, Mbio; Jørgensen *et al.* 2017, Vet Micro

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Criticism

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  - Size and composition
  - Transcpriptome

Cornforth *et al.* 2018, Proc Natl Acad Sci

Antibiotic susceptibilities of biofilm producing bacteria isolated from horse wounds

Questions or comments?

How to stitch a turkey?

Special article

Special article

Investigation of the best suture pattern to close a stuffed Christmas turkey

D. Verwilghen, V. Busoni, G. van Galen, M. Wilke

Verwilghen *et al.* 2011, Vet Rec

How to stitch a turkey?

Methods

- 15 bio bred 7 weeks old turkeys, 3.56 kg

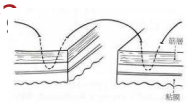
How to stitch a turkey?

Methods

- 15 bio bred 7 weeks old turkeys, 3.56 kg

- Deboned
  - Careful incision in dorsal midline
  - Flesh and skin cut from carcase + blunt dissection
  - Cavity filled with stuffing until tensionless apposition

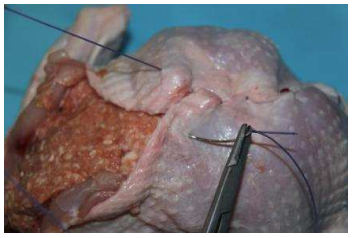
How to stitch a turkey?

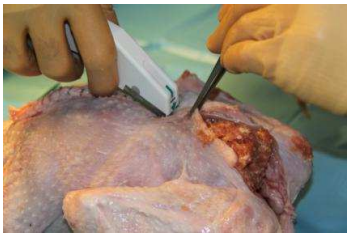


Methods

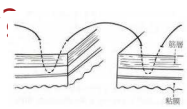
- Suture
  - Number 2 polyglactin 910 (Vicryl; Ethicon), round needle

- Lembert
- Cushing
- Skin staples
- Simple continuous
- Utrecht pattern





How to stitch a turkey?



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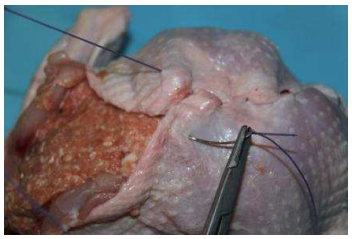

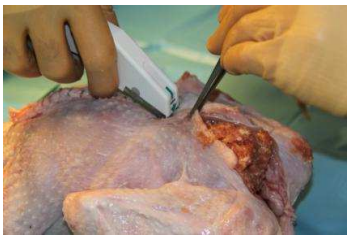

- Hygienic conditions were maintained during the whole procedure

How to stitch a turkey?

Methods

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  - 180°C for approximately two hours
  - Until internal stuffing temperature reached 75°C

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  - Disruption of skin (grade 0-3, 3=extensive disruption)
  - Cosmetic appearance (grade 1-5, 5=excellent)

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- Statistics: Analysis of variance, significance level was set at $p<0.01$
- All cooked turkeys were offered to charity on completion

How to stitch a turkey?

Results

TABLE 1: Mean skin disruption scores† and cosmetic grades‡ for stuffed turkeys

Closure pattern (n=3 per group)	Before cooking		After cooking/before suture removal		After suture removal	
	Skin disruption	Cosmetic grade	Skin disruption	Cosmetic grade	Skin disruption	Cosmetic grade
Lembert pattern	0	3.6*	0	3	2.3	2.3
Cushing pattern	0	4	0	3.6	2.6	2.3
Utrecht pattern	0	4.6	0	4.6	2.3	2.3
Simple continuous	1.3*	2*	2.3*	2	2.6	1.3
Skin staples	0	5*	0	4.6	0.3	4.6*

* Significantly different, P<0.01
† Scale 0 to 3, ranging from no to extensive disruption, ‡ Scale 1 to 5, ranging from bad to excellent

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How to stitch a turkey?

Results

How to stitch a turkey?

Conclusion

- Best Christmas helper

Merry Christmas from VWHA

Veterinary Wound Healing Association

VWHA.NET