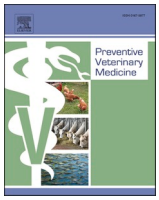
Preventive Veterinary Medicine 193 (2021) 105406

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed



Latent class analysis to assess whole-genome sequencing versus broth microdilution for monitoring antimicrobial resistance in livestock

Ayla Hesp [a,b,*], Kees Veldman [a], Michael S.M. Brouwer [a], Jaap A. Wagenaar [a,b], Dik Mevius [a,b], Gerdien van Schaik [c,d]

[a] Department of Bacteriology, Host Pathogen Interaction and Diagnostics Development, Wageningen Bioveterinary Research, Lelystad, the Netherlands
[b] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[c] Royal GD, Deventer, the Netherlands
[d] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

ARTICLE INFO

Keywords:
Antimicrobial resistance
Monitoring
Surveillance
Livestock
Bayesian
Whole-genome sequencing
Broth microdilution
Escherichia coli

ABSTRACT

Antimicrobial resistance (AMR) monitoring in animals is performed in commensal *Escherichia coli*, and other microorganisms relevant for human or veterinary health. Due to advances in the field and major reductions in cost, it is expected that whole-genome sequencing (WGS)-based antimicrobial susceptibility testing (AST) will (partly) replace culture-based AST. So far, no studies have been performed without using culture-based AST as the gold standard. Our aim was to use Bayesian latent class analysis to evaluate the accuracy of susceptibility testing of commensal *E. coli* by WGS-based AST versus culture-based AST as this test does not assume a gold standard. OpenBUGS was used to model two independent tests in three animal populations (N = 150, 50 bacterial isolates per population): veal calves, pigs, and broilers. This resulted in the first estimation of sensitivity and specificity of WGS-based AST versus culture-based AST to detect AMR without a gold standard. Both methods had high sensitivity (>0.92, lowest limit probability interval: 0.76) and specificity was generally high for both methods for all antimicrobial classes except for aminoglycosides and macrolides. We compared WGS results for different length and identity settings (%) of gene alignment and found few differences between the 60/90, 90/90 and 95/95 settings. We recommend to further investigate sensitivity and specificity of WGS-based AST by means of latent class analysis, especially for low-prevalent resistance.

1. Introduction

As part of global efforts to control antimicrobial resistance (AMR) (O'Neill, 2016), monitoring AMR in animals is performed in sentinel organisms such as commensal *Escherichia coli* (Frimodt-Moller, 2004; EFSA et al., 2019). Currently, this is mostly done with culture-based antimicrobial susceptibility testing (AST) method broth microdilution, determining minimum inhibitory concentrations (MIC) for pre-defined panels of antimicrobials. Epidemiological cut-off values (ECOFFs) or clinical breakpoints are used to determine if bacterial isolates have non-wildtype susceptibility or resistance, respectively. Recently, whole-genome sequencing (WGS) is becoming more widely available for routine AMR monitoring, and it is the expectation that WGS will mostly replace culture-based phenotypic typing in the future (Ellington et al., 2017). This paper aims to determine the validity of WGS for AMR monitoring purposes in the commensal indicator organism *E. coli*.

In the European Union, AMR monitoring in food-borne pathogens and indicator organisms from food animals is mandatory by EU legislation (2013/652/EU), and prescribed by guidelines of the European Food Safety Authority (EFSA, 2012). As part of the recently revised EFSA guidelines (EFSA et al., 2019), WGS is implemented for monitoring of Extended Spectrum Beta-Lactamase producing *E. coli* in European member states from 2021 onwards as a first step towards the transfer to WGS-based AMR monitoring. Many studies have shown that WGS performs well in identifying acquired resistance genes and point mutations that lead to phenotypic resistance (McDermott et al., 2016; Shelburne et al., 2017; Hendriksen et al., 2019; Bortolaia et al., 2020; Mahfouz et al., 2020). Next to information on AMR genes, WGS provides additional information, which is considered to enhance AMR monitoring (McDermott et al., 2016; Hendriksen et al., 2019). WGS elucidates the

* Corresponding author at: Wageningen Bioveterinary Research, Department of Bacteriology, Host Pathogen Interaction and Diagnostics Development, PO Box 65, 8200 AB, Lelystad, the Netherlands.
E-mail address: ayla.hesp@wur.nl (A. Hesp).

https://doi.org/10.1016/j.prevetmed.2021.105406
Received 14 January 2021; Received in revised form 29 April 2021; Accepted 2 June 2021
Available online 4 June 2021

genetic relatedness of resistant strains, as well as information on virulence factors, and potentially the genetic link between AMR genes and mobile genetic elements. When these are linked, resistance genes can spread among bacteria, for example from commensal organism *E. coli* to veterinary pathogens. Therefore, information on virulence and genetic links with mobile genetic elements is relevant from a public health perspective, for zoonotic potential, and for (veterinary) clinical interest. Furthermore, WGS has other advantages over culture-based antimicrobial susceptibility typing: the potential to store sequence data indefinitely, data is easier to share with other laboratories and stakeholders, and it solves the lack of reproducibility across different laboratories described for broth microdilution (Bortolaia et al., 2020).

So far, no studies have been performed without culture-based susceptibility testing as the gold standard (Mahfouz et al., 2020). Most existing studies focus on estimating the concordance of WGS-based AST to culture-based AST (Hendriksen et al., 2019), in which an objectivity bias may exist when comparing sensitivity and specificity of WGS-based AST to these other methods. Bayesian latent class analysis enables the estimation of sensitivity and specificity of diagnostic tests without a gold standard (Johnson et al., 2019). The purpose of this work is to use Bayesian latent class analysis to evaluate the sensitivity and specificity of WGS-based AST and culture-based AST to test commensal *E. coli*.

2. Methods

2.1. Sample collection

Included in the analysis were 150 commensal *E. coli* isolates collected on broiler, pig, and veal calf farms in the Netherlands in the EFFORT project (EFFORT, 2020) from October 2014 to December 2015, 10 isolates from five farms for each animal population (Ceccarelli et al., 2020). To include the diversity of the Dutch livestock sector in the sample, the farms in EFFORT were selected by different levels of antimicrobial use on farms (low to high). Faecal isolates from individual animals were randomly collected on these farms. It was part of the EFFORT sampling protocols that all animals should be sampled as close to slaughter age as possible. The EFFORT sampling protocols are described extensively in the Supplementary material of Munk et al. (2018).

2.2. Antimicrobial susceptibility testing: WGS-based

From the 150 randomly isolated E. coli strains, bacterial DNA was isolated using the Qiagen Pure Gene kit, sequencing libraries were prepared using the Illumina TruSeq kit and sequenced with Illumina HiSeq. The average genome coverage resulted between 48.4–301 times coverage. Raw sequence data have been deposited at ENA, a list of accession numbers (EFFORT ID) is available in Supplementary Table S2. High-quality trimmed reads (BBmap, version 38.87 (2020)) were assembled using Unicycler (version 0.4.5) and screened for resistance genes using ResFinder 3.0 and PointFinder (Bortolaia et al., 2020) on a local Linux server (databases downloaded April 2020). Isolates were considered resistant by WGS-based AST conform the ResFinder 3.0 and Pointfinder definitions of resistance genes that encode resistance to specific antimicrobial classes (Bortolaia et al., 2020). Results were compared between all resistance genes belonging to the class aminoglycosides as positive for WGS-based AST, versus only the two genes that encode gentamicin resistance (*aac(3′)-IId* and *aac(3′)-IV*), to show the effect on sensitivity and specificity. Similarly, phenotypical azithromycin resistance was compared to detection of only azithromycin resistance genes (*mph*(A)) versus the complete class of resistance genes for macrolides.

WGS-based AST results were compared for different settings of gene alignment to the ResFinder 3.0 database of length and identity of the resistance genes: length 60 % and identity 90 %, length 90 % and identity 90 %, length 95 % and identity 95 %, length 99 % and identity 99 %, and length 100 % and identity 100 % (Table 2). For the latent class analysis, test results of ResFinder default settings for length/identity 60/90 were used, and cross-classified with the culture-based AST results, since the aim was to evaluate WGS-based AST for routine AMR monitoring purposes. To further investigate discordant results, the WGS-based AST results of the other length/identity settings summarized in Table 2 were scrutinized.

2.3. Antimicrobial susceptibility testing: culture-based

Culture-based AST for AMR monitoring in *E. coli* was performed using the broth micro-dilution reference method according to ISO standards (ISO 20776−1) with a fixed panel of antimicrobials relevant to human healthcare according to EU legislation and European Food Safety Authority (EFSA) guidelines (Sensititre, EUVSEC antimicrobial panel). This was performed within the EFFORT project (Ceccarelli et al., 2020). The terms ‘resistant’ and ‘resistance’ in this study refer to non-wild type susceptibility, based on epidemiological cut-off (ECOFF) values as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2019). Singular culture-based AST results were used in this latent class analysis. For a set of isolates with discordant results between culture-based AST and WGS-based AST, culture-based AST was repeated to verify the results, and identify possible explanations for discordance of the WGS-based AST results.

2.4. Bayesian latent class analysis

Counts of positive and negative isolates for resistance by WGS-based AST and culture-based AST were cross-classified in tables, per antimicrobial class (Supplementary Table S1). Latent class analysis was performed in OpenBUGS software (version 3.2.3, download September 2020). Based on the difference between the two test methodologies, it was assumed the two tests were conditionally independent. Culture-based AST detects expression of resistance genes by culturing in broth while WGS detects resistance genes in the bacterial genome. A model was used comparing two independent tests in three animal populations (50 isolates for each animal population) that differed in expected prevalence of AMR. Code for the OpenBUGS model was adapted from a previous publication (Johnson et al., 2019). In all models, 1000 iterations were used as burn-in and discarded, and summary statistics were based on the next 10,000 iterations. Convergence of each model was assessed by standard diagnostic procedure for latent class analysis (Benedict et al., 2014).

2.5. Prior probability distributions

The prior probability distributions of resistance prevalence in the three animal populations were based on data from the Dutch National monitoring program (MARAN) in which culture-based AST is performed (MARAN et al., 2016). The dataset of MARAN is large (300 commensal *E. coli* isolates per animal species/year) and consists of isolates from random samples of animals at slaughter. Given that sample selection differed for MARAN (random representative) and our samples (10 random animals in five selected herds), weak-informative priors for resistance prevalence were used similar to the methodology of Benedict et al. (2014). The priors were based on the MARAN data of 2015 (MARAN et al., 2016) for each antimicrobial class in broilers, veal calves, and pigs. Beta distributions (Table 1) were calculated with Betabuster 1.0 freely available software (Betabuster 1.0, accessed September 2020). For sensitivity and specificity of both tests, weak-informative priors for culture-based AST in *E. coli* (Table 1) were used from Benedict et al. (2014). In a sensitivity analysis, model results were compared with a non-informative, uniform prior distributions (Beta(1,1)) for sensitivity and specificity.

Table 1
Prior probability distributions for prevalence of antimicrobial resistance and for sensitivity and specificity of culture-based antimicrobial susceptibility testing by broth microdilution (culture-based AST) and whole-genome sequenced based AST to detect antimicrobial resistance in livestock.

Antimicrobial class		Beta distribution parameters (a, b)	Mode (%)	95 % PI[a]
Gentamicin / Aminoglycosides	Veal calves	(1.2, 25.7)	1.0	0.2-15
	Pigs	(1.2, 25.7)	1.0	0.2-15
	Broilers	(3.0, 50.1)	4.0	1.2-13
Beta-lactams (ampicillin)	Veal calves	(14.0, 60.0)	19.0	11-30
	Pigs	(22.6, 53.8)	29.0	20-40
	Broilers	(2.8, 2.6)	53.0	15-88
Phenicols	Veal calves	(5.9, 38.4)	11.5	5-24.5
	Pigs	(3.7, 26.6)	9.4	3-26
	Broilers	(5.2, 34.9)	11.0	4.6-25
Trimethoprim	Veal calves	(3.4, 17.7)	12.7	4-34
	Pigs	(5.4, 8.9)	35.9	16-63
	Broilers	(3.5, 4.5)	41.5	14-76
Azithromycin / Macrolides	Veal calves	(1.0, 21.85)	0.0	0-15
	Pigs	(1.25, 25.7)	1.0	0.2-15
	Broilers	(1.85, 34.5)	2.5	0.5-14
Quinolones	Veal calves	(2.9, 27.8)	6.7	2-22
	Pigs	(1.1, 13.0)	0.7	0.3-25
	Broilers	(47.3, 60.0)	44.0	34-54
Sulfonamides	Veal calves	(19.0, 60.2)	23.3	15-34
	Pigs	(8.0, 11.3)	40.3	21-63
	Broilers	(4.5,4.9)	47.0	19-77
Tetracyclines	Veal calves	(10.2, 14.2)	41.0	23-61
	Pigs	(6.4, 7.5)	45.3	22-71
	Broilers	(27.4, 48.4)	35.8	26-47
Culture-based AST (broth microdilution)	Sensitivity	(4.8, 1.2)	83.3	43.1–99.0
	Specificity	(4.8, 1.2)	83.3	43.1–99.0
Whole-genome sequenced based AST	Sensitivity	(4.8, 1.2)	83.3	43.1–99.0
	Specificity	(4.8, 1.2)	83.3	43.1–99.0

[a] Probability interval.

Table 2
Results of culture-based antimicrobial susceptibility testing (AST) by broth microdilution versus different gene alignment settings for whole-genome sequenced based AST to detect antimicrobial resistance in livestock (N = 150).

Antimicrobial class	MIC[a]	60/90[b]	90/90[c]	95/95[d]	99/99[e]	100/100[f]
Gentamicin	2	1	0	0	0	0
Beta-lactams	76	77	76	76	75	66
Phenicols	22	22	21	21	14	0
Trimethoprim	64	67	67	67	67	18
Azithromycin	3	3	3	3	3	3
Quinolones	22	23	23	23	19	2
Sulfonamides	74	75	75	75	73	70
Tetracyclines	94	94	94	94	94	79

[a] Number of isolates found resistant by broth microdilution (MIC) out of a total of 150 isolates.
[b] Number of isolates found resistant by WGS (N = 150) with length/identity setting 60/90 % for the alignment.
[c] Number of isolates found resistant by WGS (N = 150) with length/identity setting 90/90 %.
[d] Number of isolates found resistant by WGS (N = 150) with length/identity setting 95/95 %.
[e] Number of isolates found resistant by WGS (N = 150) with length/identity setting 99/99 %.
[f] Number of isolates found resistant by WGS (N = 150) with length/identity setting 100/100 %.

3. Results

In this study a latent class model was used to determine the sensitivity and specificity of WGS-based AST versus culture-based AST without a gold standard. Convergence of the latent class model was good, based on history and auto-correlation plots (examples for gentamicin, beta-lactams and phenicols presented in Supplementary Figure S1). Model results showed that, in this data, the sensitivity and specificity of WGS-based AST and culture-based AST were similar. This corresponded to the cross-classified test outcomes of WGS-based AST and culture-based AST, in which relatively few differences were found (Supplementary Table S1). For tetracyclines, test results were identical for WGS-based AST and culture-based AST. For the other antimicrobial classes, only a small number of isolates (n = 13) were found to be discordant between the two tests (Table 4, Table S1). Discordance was much higher for the complete classes of aminoglycosides and macrolides if all genes which encode resistance to any aminoglycoside or macrolide were considered as positive for resistance (Table S1).

Results of the comparison of WGS-AST methodology regarding different settings for the gene alignment are presented in Table 2. The differences between the settings 60/90, 90/90, and 95/95 were few (Table 2). The highest number of resistant isolates (both with culture-based AST and WGS-based AST with length/ID: 60/90 respectively) were found for tetracyclines (n = 94, n = 94), sulfonamides (n = 74, n = 75), trimethoprim (n = 64, n = 67) and beta-lactams (n = 76, n = 77) (Table 2). Lower numbers of resistant isolates were identified for quinolones (n = 22, n = 23) (Table 2). Resistance for gentamicin (n = 2, n = 1) and azithromycin (n = 3, n = 3) was rarely detected (Table 2). Overall, the difference between culture-based AST and WGS-based AST (length/ID: 60/90) was small (Table 2). The difference remained small with more strict settings, but substantially increased when using the 100/100 settings (Table 2).

Regarding the latent class analysis results as shown in Table 3, estimated prevalence was low for gentamicin with 1%, 2% and 2% in veal calves, pigs, and broilers, respectively. Azithromycin resistance amounted 5%, 2% and 2% in veal calves, pigs, and broilers, respectively (Table 3). For sulfonamides, prevalence was moderate in veal calves (26 %) and low in pigs (6%) and broilers (4%) (Table 3). For both culture-based AST and WGS-based AST, the sensitivity and specificity for most antimicrobial classes was high, with the exception of sensitivity of the complete class of aminoglycosides and gentamicin, and the complete class of macrolides and azithromycin (Table 3). For all other antimicrobial classes, the sensitivity was >0.92 (lowest probability interval limit: 0.76) and the specificity was generally high for both WGS-based AST and culture-based AST (Table 3).

In case of discordant results, culture-based AST was repeated (Table 4). For most isolates (n = 10), resistance found was identical to the first test, with exception of three isolates. Isolates initially tested resistant for gentamicin (n = 2) or azithromycin (n = 1) were found susceptible after repeating the test (Table 4). These results were then concordant with WGS-based AST.

Scrutinizing the other length/identity settings of the WGS-based AST results clarified more discordant results (Tables 2, 4). For phenicols, for example, one *catA1* gene was not detected with higher length/identity setting i.e. 90/90 (Table 2). Another discordant isolate with a *floR* gene was still found positive for this gene with a setting of 95/95, but not anymore with settings of 99/99. For trimethoprim resistance, resistance genes in the three discordant isolates were not found with length/identity of 100/100, corresponding with the repeated culture-based AST (Table 2). The isolate with a *aac(3′)IId* gene was detected with 60/90 but not detected with the 90/90 alignment setting (Tables 2, 4).

4. Discussion

The purpose of this study was to evaluate the sensitivity and specificity of WGS-based AST versus culture-based AST to monitor AMR in

Table 3
Latent class analysis estimates (median and 95 % probability interval) for sensitivity and specificity of culture-based antimicrobial susceptibility testing (AST) versus whole-genome sequenced based AST to detect antimicrobial resistance in veal calves (n = 50), pigs (n = 50) and broilers (n = 50).

Antimicrobial class	Prevalence[a]			Sensitivity[b]		Specificity[c]	
	Veal calves	Pigs	Broilers	Culture-based AST	WGS- based AST	Culture-based AST	WGS- based AST
Gentamicin	0.01	0.02	0.03	0.77 (0.36–0.98)	0.76 (0.35–1.00)	0.98 (0.95–1.00)	0.99 (0.97–1.00)
Aminoglycosides	0.02	0.03	0.03	0.73 (0.29–0.98)	0.79 (0.40–0.98)	0.99 (0.96–1.00)	0.46 (0.38–0.54)
Beta-lactams (ampicillin)	0.34	0.29	0.67	0.99 (0.94–1.00)	0.99 (0.95–1.00)	0.99 (0.95–1.00)	0.98 (0.93–1.00)
Phenicols	0.45	0.24	0.53	0.92 (0.76–0.99)	0.92 (0.76–0.99)	0.99 (0.95–1.00)	0.99 (0.95–1.00)
Trimethoprim	0.08	0.03	0.41	0.97 (0.90–1.00)	0.99 (0.94–1.00)	0.99 (0.95–1.00)	0.97 (0.92–1.00)
Azithromycin	0.03	0.04	0.04	0.84 (0.43–0.99)	0.83 (0.44–0.99)	0.83 (0.43–0.99)	0.83 (0.43–1.00)
Macrolides	0.03	0.02	0.02	0.77 (0.36–0.99)	0.88 (0.54–0.99)	0.99 (0.95–1.00)	0.07 (0.04–0.12)
Quinolones	0.38	0.34	0.55	0.96 (0.84–1.00)	0.97 (0.86–1.00)	0.99 (0.97–1.00)	0.99 (0.96–1.00)
Sulfonamides	0.25	0.08	0.08	0.98 (0.94–1.00)	0.99 (0.95–1.00)	0.99 (0.95–0.99)	0.99 (0.94–1.00)
Tetracyclines	0.70	0.55	0.40	0.99 (0.96–1.00)	0.99 (0.96–1.00)	0.98 (0.93–1.00)	0.98 (0.93–1.00)

[a] Median for estimated prevalence.
[b] Median for sensitivity, the 95 % probability intervals are listed in parenthesis.
[c] Median for specificity, the 95 % probability intervals are listed in parenthesis.

Table 4
Discordant isolates (n = 13) in results of broth microdilution (culture-based AST) versus whole-genome sequenced based AST to detect antimicrobial resistance in livestock.

Antimicrobial class	Antimicrobial	Isolate ID	MIC[a]	Repeated MIC	ECOFF	Resistance gene
Aminoglycosides	Gentamicin	100302010	4	1	2	None for gentamicin
		101702014	8	2		None for gentamicin
		103003004	1	0.5		*aac(3′)-IId*
Beta-lactams	Ampicillin	110704022	2	2	8	bla_{TEM-1C}
Phenicols	Chloramphenicol	110004010	32	32	16	None for phenicols
		110004014	32	32		None for phenicols
		110504004	8	8		*catA1*
		111604014	8	8		*floR*
Trimethoprim	Trimethoprim	110504004	0.5	0.5	2	*dfrA1*
		111604014	0.25	0.25		*dfrA1*
		111804010	0.25	0.25		*dfrA7*
Macrolides	Azithromycin	110504020	8	8	16	*mph*(A), *mph*(B)
		102702012	128	8		None for azithromycin
Quinolones	Ciprofloxacin	102302012	0.015	0.015	2	*parC* p.A56T
Sulfonamides	Sulfamethoxazole	111604014	8	8	16	*sul1*, *sul2*

[a] Minimum inhibitory concentration (MIC) determined by broth microdilution (culture-based AST).

livestock, without a gold standard, by means of latent class analysis. The estimated sensitivity and specificity across antimicrobial classes are similar for WGS-based AST and culture-based AST. The latent class analysis allowed the test validity of both tests to be determined relative to the latent class, the true resistance for antimicrobials.

4.1. Test validity

For some antimicrobial classes, the sensitivity of WGS-based AST is slightly higher than of culture-based AST, although probability intervals overlap (Table 3). Also, there is some indication that the overall specificity of WGS-based AST is lower than of culture-based AST, but these probability intervals also overlap, indicating that the specificity of both methods is similar. Few differences were found in the outcomes between the two methods, resulting in low numbers of discordant isolates. The finding that WGS-based AST performs at least as well as culture-based AST is in line with previous studies using WGS-based AST as the gold standard. The review paper of Hendriksen et al. (2019) includes an overview of WGS-based AST versus culture-based AST comparisons, showing that many studies report high concordance of WGS-based AST and culture-based AST. A study by McDermott et al. (2016) in Salmonella from retail meat reached similar conclusions as this study, reporting high sensitivity and specificity for WGS-based AST.

4.2. Advantages and disadvantages of culture-based AST and WGS-based AST

The advantage of culture-based AST is that the phenotype is measured (Ellington et al., 2017) as a cumulative result of all resistance mechanisms present in a bacterial cell. For example, less specific resistance mechanisms like efflux pumps leading to resistance to multiple antimicrobial classes (Swick et al., 2011). A limitation of broth microdilution is the lack of reproducibility of end-point-reading (Bortolaia et al., 2020; Mahfouz et al., 2020).

In WGS-based AST, the database used determines the outcomes, and defines strains resistant versus susceptible. The choice of database may influence the sensitivity and specificity of WGS-based AST (Mahfouz et al., 2020). We used ResFinder 3.0, considering it is well curated and performs well compared to other resistance databases (Hendriksen et al., 2019; Mahfouz et al., 2020). The results presented here and previously by McDermott et al. (2016) show that sensitivity and specificity of WGS-based AST versus culture-based AST is mostly antimicrobial class specific and not so much database specific. Therefore, we expect that re-analysis using for instance ResFinder 4.0 or CARD will not result in major differences in the estimated sensitivity and specificity of WGS-based AST.

The comparison of the aminoglycoside and macrolide antimicrobial classes illustrates the importance of the definition of specific resistance phenotypes of AMR genes in the interpretation of WGS-based AST. Aminoglycosides are represented in culture-based AST by gentamicin but many aminoglycoside resistance genes do not lead to gentamicin

resistance, potentially leading to a high number of false-positive results when this distinction is not made (specificity of WGS: 0.46, Table 3). Similarly for macrolides, the efflux pump encoding gene, *mdfA* (Edgar and Bibi, 1997) does not always lead to phenotypic azithromycin resistance, resulting in a high number of false positives (specificity of WGS: 0.07, Table 3).

4.3. Explanation of discordant results

For almost all discordant results, we identified the cause of the mismatch. Some isolates were found resistant to culture-based AST, without detection of specific resistance mechanisms by WGS-based AST (Table 4). In three cases the causes were 'skips' or other issues with reproducibility of MICs, resulting in a match between culture-based and WGS-based AST after repeating the MIC. The test results repetitively susceptible to culture-based AST despite being resistant by WGS-based AST (Tables 4, and S1) are partly explained by the relatively low length/ID settings of 60/90 used for ResFinder (Table 2). Genes could have mutations, and may therefore not be expressed as phenotypic resistance (Bortolaia et al., 2019). In a systematic review, Mahfouz et al. (2020) discuss that it may be advisable to revise the default settings for ResFinder of 60/90 length/identity. In our data, only minor differences were found between the 60/90, 90/90 and 95/95 settings (Table 2). In general, the 60/90 setting seems well suited for routine AMR monitoring, although in some cases resistance genes are identified which do not lead to phenotypic expression (Table 2). Using higher-length settings then prevents a false-negative result. AMR genes can also be detected in raw sequence data instead of assemblies, it is expected this will not influence the estimated sensitivity and specificity.

For some discordant isolates we found a very low sequence depth as the cause, possibly due to contamination or spill-over between multiplexed samples. The standard depth-filter of Unicycler is 25 % sequence depth compared to the chromosomal sequence depth, but this was turned off for these assemblies, as some plasmid encoded resistance genes were previously missed due to this depth-filter. For routine AMR monitoring, a setting between 10 and 25 % is advisable, to prevent false-positive findings.

Two phenicol resistant isolates in veal calves are rare examples for which we did not find an explanation for the difference in test outcomes (Table 4). This may be the rare situation where the detected phenotype concerns new (variations of) resistance genes, or results could be different using a different database.

4.4. Assumptions of latent class analysis

The latent class model estimates the true resistance prevalence by combining the data with the prior information and estimating how both tests identify the true resistance prevalence in the different animal populations (Johnson et. al 2019). Consequently, the prevalence of resistance in the data will influence the precision of the estimated sensitivity and specificity. Gentamicin and azithromycin resistance both have a low prevalence in all three animal populations (Table S1) and in the prior information (Table 1). This results in lower estimates of sensitivity (with wider probability intervals) for those two antimicrobial classes of both culture-based and WGS-based AST (Table 3). Interestingly, in another study with culture-based AST as the gold standard for WGS-based AST, also a lower sensitivity of WGS-based AST for gentamicin resistance (0.93) than for other antimicrobial classes was found (McDermott et al., 2016). To evaluate our findings, this analysis should be repeated in populations where resistance prevalence is higher (although this will be difficult due to the general low prevalence of these resistance mechanisms in *E. coli*) or with more data, should these become available.

In this latent class model, it was assumed that resistance prevalence differs in the different animal populations. However, for some antimicrobials, the prevalence was almost equal in the three populations, which potentially affects the accuracy of the sensitivity and specificity estimates. Others investigated the impact of breaching the prevalence assumption and found that for tests with high sensitivity or specificity this was of little influence (Toft et al., 2005).

The sample size in our study was relatively small (10 isolates from five farms) and resistance prevalence was low for some antimicrobial classes. Latent class analyses can deal with such limitations as long as there are sufficient degrees of freedom to estimate the posterior distributions of the parameters (Johnson et al., 2019). The models for the different antimicrobials all converged fairly rapidly (Supplementary Figure S1). This is presumably because an important requirement for test validation was met: all results were generated in the same laboratory with experienced staff and a high level of standardization. To improve external validity, it is advised to repeat this analysis with more, and preferably less clustered data.

4.5. Conclusions for AMR monitoring purposes

From the results of this Bayesian latent class analysis, we conclude that WGS-based AST is just as suitable for monitoring AMR in livestock as culture-based AST. Our findings highlighted some genetic variation of resistance genes and their phenotypic expression, compared to traditional AMR monitoring generated by culture-based AST. This can be of aid in future interpretation, when WGS will be further implemented to monitor AMR in livestock.

Declaration of Competing Interest

None.

Acknowledgements

We thank the Ministry of Agriculture, Nature and Food Quality for funding the WOT-O project (WOT-01-002-003) for the sequencing of the bacterial isolates, and the EFFORT consortium for funding the susceptibility testing of the bacterial strains. Roosmarijn Luiken and Liese van Gompel we would like to thank for organizing the sampling of the EFFORT strains. We thankfully acknowledge Yvon Geurts, Alieda van Essen, Joop Testerink, Marga Japing and Albert de Boer for their technical assistance. We also acknowledge the contribution of Jeanet van der Goot to this work and Daniela Ceccarelli for analysing the MIC dataset within EFFORT, and Haitske Graveland for the project management within EFFORT. Finally, we would like to thank Bas Engel for his aid in optimizing the OpenBUGS coding.

Funding

This research was supported by the Ministry of Agriculture, Nature and Food Quality (WOT-01-002-003) and Wageningen Bioveterinary Research. The susceptibility testing of the bacterial strains was part of the Ecology from Farm to Fork Of microbial drug Resistance and Transmission (EFFORT) project, funded by the European Commission, 7th Framework Program for Research and Innovation (FP7-KBBE-2013–7, grant agreement: 613754).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2021.105406.

References

BBmap, 2020. BBMap Short Read Aligner, and Other Bioinformatic Tools (accessed April 2020. https://sourceforge.net/projects/bbmap/.

Benedict, K.M., Gow, S.P., Reid-Smith, R.J., Booker, C.W., McAllister, T.A., Morley, P.S., 2014. Latent class comparison of test accuracy when evaluating antimicrobial

susceptibility using disk diffusion and broth microdilution to test Escherichia coli and Mannheimia haemolytica isolates recovered from beef feedlot cattle. Epidemiol. Infect. 142, 2314–2325.

Bortolaia, V., Kaas, R.S., Ruppe, E., Roberts, M.C., Schwarz, S., Cattoir, V., Philippon, A., Allesoe, R.L., Rebelo, A.R., Florensa, A.F., Fagelhauer, L., Chakraborty, T., Neumann, B., Werner, G., Bender, J.K., Stingl, K., Nguyen, M., Coppens, J., Xavier, B. B., Malhotra-Kumar, S., Westh, H., Pinholt, M., Anjum, M.F., Duggett, N.A., Kempf, I., Nykäsenoja, S., Olkkola, S., Wieczorek, K., Amaro, A., Clemente, L., Mossong, J., Losch, S., Ragimbeau, C., Lund, O., Aarestrup, F.M., 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J. Antimicrob. Chemother.

Ceccarelli, D., Hesp, A., van der Goot, J., Joosten, P., Sarrazin, S., Wagenaar, J.A., Dewulf, J., Mevius, D.J., Effort Consortium, O, 2020. Antimicrobial resistance prevalence in commensal Escherichia coli from broilers, fattening turkeys, fattening pigs and veal calves in European countries and association with antimicrobial usage at country level. J. Med. Microbiol. 69, 537–547.

Edgar, R., Bibi, E., 1997. MdfA, an Escherichia coli multidrug resistance protein with an extraordinarily broad spectrum of drug recognition. J. Bacteriol. 179, 2274–2280.

EFFORT, 2020. Ecology from Farm to Fork of Microbial Drug Resistance and Transmission (accessed December 14, 2020. http://www.effort-against-amr.eu/.

EFSA, 2012. Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in Salmonella, Campylobacter and indicator Escherichia coli and Enterococcus spp. Bacteria transmitted through food. Efsa J. 10 (6), 2742. https://doi.org/10.2903/j.efsa.2012.2742, 2012 [64 pp.] Available online: www.efsa.europa.eu/efsajournal.

EFSA, Aerts, M., Battisti, A., Hendriksen, R., Kempf, I., Teale, C., Tenhagen, B.-A., Veldman, K., Wasyl, D., Guerra, B., Liébana, E., Thomas-López, D., Belœil, P.-A., 2019. Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. EFSA J. 17, e05709. Available online: www.efsa.europa.eu/efsajournal.

Ellington, M.J., Ekelund, O., Aarestrup, F.M., Canton, R., Doumith, M., Giske, C., Grundman, H., Hasman, H., Holden, M.T.G., Hopkins, K.L., Iredell, J., Kahlmeter, G., Köser, C.U., MacGowan, A., Mevius, D., Mulvey, M., Naas, T., Peto, T., Rolain, J.M., Samuelsen, Ø., Woodford, N., 2017. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin. Microbiol. Infect. 23, 2–22.

EUCAST, 2019. Antimicrobial Wild Type Distributions of Microorganisms (accessed December 14, 2020. https://eucast.org/mic_distributions_and_ecoffs/.

Frimodt-Moller, N., 2004. Microbial threat–The Copenhagen recommendations initiative of the EU. J. Vet. Med. B Infect. Dis. Vet. Public Health 51, 400–402.

Hendriksen, R.S., Bortolaia, V., Tate, H., Tyson, G.H., Aarestrup, F.M., McDermott, P.F., 2019. Using genomics to track global antimicrobial resistance. Front. Public Health 7, 242.

Johnson, W.O., Jones, G., Gardner, I.A., 2019. Gold standards are out and Bayes is in: implementing the cure for imperfect reference tests in diagnostic accuracy studies. Prev. Vet. Med. 167, 113–127.

Mahfouz, N., Ferreira, I., Beisken, S., von Haeseler, A., Posch, A.E., 2020. Large-scale assessment of antimicrobial resistance marker databases for genetic phenotype prediction: a systematic review. J. Antimicrob. Chemother. 75, 3099–3108.

MARAN, Veldman, K., Mevius, D.J., Wit, B., Van Pelt, W., Heederik, D., 2016. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2015.

McDermott, P.F., Tyson, G.H., Kabera, C., Chen, Y., Li, C., Folster, J.P., Ayers, S.L., Lam, C., Tate, H.P., Zhao, S., 2016. Whole-genome sequencing for detecting antimicrobial resistance in nontyphoidal Salmonella. Antimicrob. Agents Chemother. 60, 5515–5520.

Munk, P., Knudsen, B.E., Lukjancenko, O., Duarte, A.S.R., Van Gompel, L., Luiken, R.E. C., Smit, L.A.M., Schmitt, H., Garcia, A.D., Hansen, R.B., Petersen, T.N., Bossers, A., Ruppé, E., Lund, O., Hald, T., Pamp, S.J., Vigre, H., Heederik, D., Wagenaar, J.A., Mevius, D., Aarestrup, F.M., 2018. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. Nat. Microbiol. 3, 898–908.

O'Neill, 2016. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance. Chaired by Jim O'Neill. Available online: https://amr-review.org/.

Shelburne, S.A., Kim, J., Munita, J.M., Sahasrabhojane, P., Shields, R.K., Press, E.G., Li, X., Arias, C.A., Cantarel, B., Jiang, Y., Kim, M.S., Aitken, S.L., Greenberg, D.E., 2017. Whole-genome sequencing accurately identifies resistance to extended-spectrum β-Lactams for major gram-negative bacterial pathogens. Clin. Infect. Dis. 65, 738–745.

Swick, M.C., Morgan-Linnell, S.K., Carlson, K.M., Zechiedrich, L., 2011. Expression of multidrug efflux pump genes acrAB-tolC, mdfA, and norE in Escherichia coli clinical isolates as a function of fluoroquinolone and multidrug resistance. Antimicrob. Agents Chemother. 55, 921–924.

Toft, N., Jørgensen, E., Højsgaard, S., 2005. Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. Prev. Vet. Med. 68, 19–33.