The EQuAfrica Pilot programme was launched to evaluate processes and procedures for the implementation of the EQuAfrica External Quality Assessment (EQA) for Antimicrobial Resistance (AMR) testing with “One Health” approach.

Participating laboratories, including human and animal health as well as environmental laboratories, were enrolled in the EQuAfrica online system. Prepared EQA panels were shipped to the 67 enrolled participants. The official cycle open date was 10 May 2021 and closing date for result submission was 24 May 2021. Due to delays in the delivery of some of the shipments, participants communicating they had received their panels late were granted additional time for processing and submitting their results. For these participants the closing date for submission of results was extended to 01 June 2021.

	Number of participants	%
Received EQA samples	67	100%
Declined participation - reason provided	3	4.5%
Results received	51	76%
No results submitted	13	19.5%

Table 1. Breakdown of participant numbers for EQuAfrica Pilot programme

Criteria and terminology used in evaluation of results

All graded areas require 80% referee consensus before being evaluated. Based on specimen type and clinical details participants were asked to perform culturing and report on microscopy, serotyping/serogrouping, final organism identification and antibiotic susceptibility testing.

Assessment criteria:

- Acceptable scores – Score of 4 or 3
- Unacceptable scores – Score of 1 or 0
- Not evaluated (NE) – Assigned if a valid reason for not submitting a response for a specific graded area is provided.
- Overall acceptable target – percentage of all participants reporting correct responses per graded area. Overall target is 80%.

Quality control, homogeneity and stability of survey samples

Isolates were tested in the facilitating EQA laboratory to confirm the expected results before shipment preparation and again at the close of the survey to confirm that there were no changes in the expected results. All samples were cultured weekly in the facilitating EQA laboratory and remained viable, stable and uncontaminated until the closing of the survey. Quality control results of all samples were acceptable prior to assessment of participants’ results.

Table 2. Sample information

Sample number	Date of sample preparation	Sample type	Referee consensus
Sample A	04/04/2021	Lyophilised isolate	Yes*
Sample B	09/04/2021	Lyophilised isolate	Yes*
Sample C	07/04/2021	Lyophilised isolate	Yes*
Sample D	07/04/2021	Lyophilised isolate	Yes*
Sample E	19/04/2021	Lyophilised isolate	Yes

*Samples evaluated for AST, all evaluated antibiotics had ≥80% referee consensus.

Sample A

	Human health	Animal health
Clinical information	A cerebrospinal fluid sample collected from an 8-day-old male baby with meningitis.	A farm encountered an outbreak with peracute death in healthy, well-conditioned, recently weaned pigs. The pigs suffered from loss of coordination, periocular oedema and extensive oedema of the stomach and mesocolon. Diarrhoea preceded the signs of oedema disease.
Sample type	Cerebrospinal fluid	Small intestine tissue from a pig

Participants were asked to report on microscopy, identification and antimicrobial susceptibility testing for the pathogen isolated. The list of antimicrobial agents to be tested was provided. The sample contained *Escherichia coli* displaying ampicillin resistance due to plasmid-mediated TEM-1 β-lactamase production.
Participants performing serotyping on the *E. coli* isolated were not penalised when reporting the final identification.
An overview of participant's performance is shown below.

Sample A - Overview of Microscopy results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**48**	**96%**
	Gram-negative bacilli	47	
	Gram-negative cocco-bacilli	1	
1	**Correct gram stain and incorrect morphology results:**	**1**	**2%**
	Gram-negative cocci	1	
0	**Incorrect gram stain and morphology results:**	**1**	**2%**
	Gram-positive cocci	1	
NE	Not applicable	1	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Sample A - Overview of Final identification results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**47**	**96%**
	Escherichia coli	44	
	Escherichia coli - Escherichia coli K1	1	
	Enterohaemorrhagic *E. coli*	1	
	Enteropathogenic *E. coli* (Pool A)	1	
3	**Correct genus, no species specified:**	**1**	**2%**
	Escherichia species	1	
1	***Escherichia* species other than coli / Unnamed/unspecified micro-organism:**	**1**	**2%**
	A Gram-negative bacillus	1	
NE	Not applicable	**2**	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Identification methods used included conventional methods (method based on cultivation procedures and manual biochemical identification), identification test kits e.g. API, automated methods and a combination of conventional methods with other methods. It is important to correlate results of basic biochemistry with the final identification of the organism irrespective of other methods used. Oxidase and indole are tests that assist with the identification of isolates belonging to the *Enterobacterales* family and have been reported by a number of participants.

Table 3. Methods used for identification for Sample A with scores achieved

Methods used for organism identification	Number of participants	Scores achieved per method used			
		4	3	1	0
API	7	7			
Conventional methods only	26	24	1	1	
MALDI-TOF	4	4			
Molecular method and MALDI-TOF	1	1			
Phoenix	2	2			
Vitek systems	4	4			
Conventional methods with API	1	1			
Conventional methods with Biolog	1	1			
Conventional methods with Phoenix	1	1			
Conventional methods with Phoenix & MALDI-TOF	1	1			

Forty-eight participants were evaluated for antimicrobial susceptibility testing. Two participants provided reasons for not reporting AST results and were not evaluated, one participant did not report any of the requested antimicrobial agents and was not evaluated. Only requested antimicrobial agents were assessed. Additional information for guidelines and test methods used by participants for AST are shown in table 4, although not evaluated, Extended spectrum β-lactamase (ESBL) results submitted by participants have been included in Table 4. An overview of Sample A AST results is shown in Figure 1.

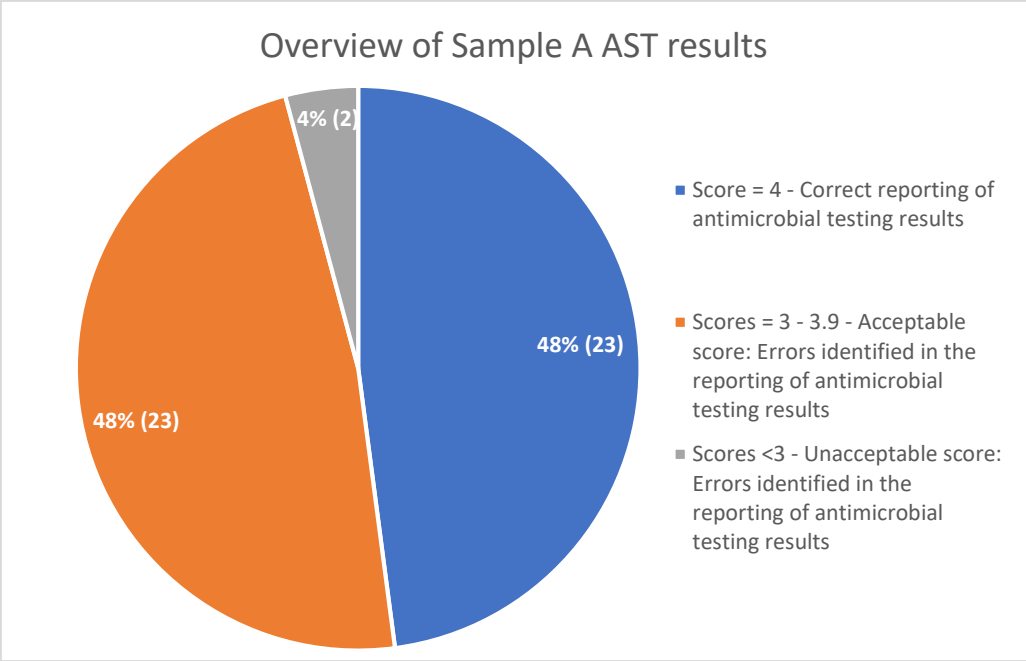


Figure 1. Overview of participant AST scores for Sample A. Not evaluated participants are excluded from the denominator when calculating percentages

Table 4. Guidelines and test methods used by evaluated participants for Sample A AST results.

Criteria	Number of participants
Guidelines used:	
EUCAST	4
CA-SFM	6
CLSI	36
CLSI & EUCAST	1
Unknown guideline	1
AMR test method used:	
Kirby-Bauer disk method	38
Kirby-Bauer disk method and a MIC method	2
MIC only	8
ESBL reported:	
Positive	2
Negative	8

Table 5: Summary of antimicrobial susceptibility testing results for Sample A showing categorical agreement (NB: Excluding participants with misidentification of the isolate and those who did not provide an interpretation for their entry)

Requested Antimicrobial agent	Participants performing Disk susceptibility testing						Participants performing MIC testing					
	Number of participants testing	R	I	S	%Acceptable	Acceptable response	Number of participants testing	R	I	S	%Acceptable	Acceptable response
Ampicillin	33	31	1	1	94	R	9	9	0	0	100	R
Amoxicillin-clavulanic acid	26	10	5	11	42	S	8	0	1	7	88	S
Cefepime	22	1	0	21	95	S	7	0	0	7	100	S
Cefotaxime	23	2	1	20	87	S	5	0	1	4	80	S
Cefoxitin	23	0	0	23	100	S	4	0	0	4	100	S
Ceftazidime	25	2	1	22	88	S	6	0	1	5	83	S
Ceftriaxone	31	2	2	27	87	S	7	0	0	7	100	S
Amikacin	25	1	0	24	96	S	7	0	0	7	100	S
Gentamicin	30	1	0	29	97	S	9	0	0	9	100	S
Tobramycin	12	1	0	11	92	S	3	0	0	3	100	S
Ciprofloxacin	34	4	2	27	79	S	6	0	0	6	100	S
Ertapenem	17	1	0	16	94	S	6	0	0	6	100	S
Imipenem	19	1	0	18	95	S	3	0	0	3	100	S
Meropenem	30	0	0	30	100	S	7	0	0	7	100	S
Piperacillin/Tazobactam	14	2	1	11	79	S	5	0	0	5	100	S
Trimethoprim/sulfamethoxazole	31	3	0	28	90	S	8	0	0	8	100	S

mE = Minor error
ME = Major error
VME = Very major error

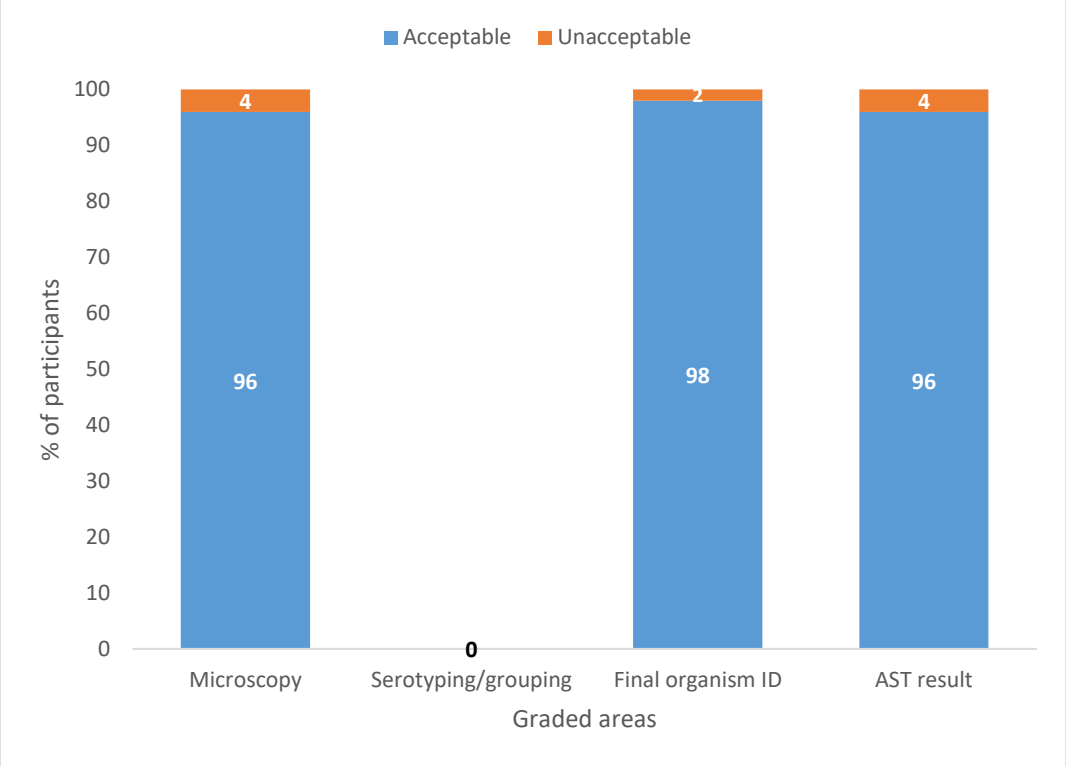


Figure 2. Summary of results for sample A shown as percentages of acceptable and unacceptable scores

Discussion

E. coli is the bacterial species most commonly recovered in the clinical laboratories and has been incriminated in infectious diseases involving virtually every human tissue and organ system. *E. coli* is one of the most common organisms involved in gram-negative sepsis and endotoxin-induced shock. Urinary tract and wound infections, pneumonia in immunosuppressed hospitalised patients, and meningitis in neonates are other common infections caused by *E. coli*. This organism with virulence factors causes community and hospital associated infections.[2]

Acceptable microscopy results were reported by 96% (n=48) of participants.

For identification of the organism for sample A, 96% (n=48) of participants reported acceptable responses. Of these, 2% (n=1) of participants correctly identified the organism to the genus level only and 94% (n=47) were able to correctly identify the organism to the species level i.e. *Escherichia coli*. An incomplete organism identification was reported by 2% (n=1) of participants.

Acceptable scores for antimicrobial susceptibility testing were achieved by 96% (n=48) of participants. Errors identified in AST results for participants reporting using disk susceptibility and MIC methods are shown in Table 5 above. Majority of the errors in AST result received were observed in participants submitting disk susceptibility testing results with only three minor errors observed in MIC results received.

The isolate possess the *bla*TEM-1B gene displaying ampicillin resistance. Of the 42 participants reporting results for ampicillin, one participant using disk susceptibility testing, reported it as susceptible resulting in a very major error (VME) in testing.

Of note, sixteen errors for amoxicillin-clavulanic acid results were submitted. Ten major errors and five minor errors were observed in participants reporting using the disk susceptibility testing method and one minor error for participants reporting using an MIC method.

Sample B

	Human health	Animal health
Clinical information	A 32-year-old female, presented with fever and chest crackles and on X-ray showing multiple pulmonary abscesses.	A routine visit by the veterinarian due to occasional cases of abscesses in a very few pigs. In general, no clinical signs were observed in the pig farm.
Sample type	Broncho-alveolar lavage	Nasal swab from a pig

Participants were requested to report on microscopy, identification and antimicrobial susceptibility testing for the pathogen isolated. The list of antimicrobial agents to be tested was provided. An overview of participant's performance is shown below. The sample contained a methicillin resistant *Staphylococcus aureus* (MRSA). Methicillin resistance in this isolate is due to the presence of the *mecA* gene which codes for penicillin binding proteins with low affinity for β-lactams. In addition, the isolate possess other resistance genes coding for phenotypic resistance to a number of other antibiotics, these are:

Antibiotic name	Phenotypic result	Gene present
Ampicillin	R	*blaZ*
Ciprofloxacin	R	*grlA, gyrA*
Erythromycin	R	*Msr(A), mph(C)*
Penicillin	R	*blaZ*

Sample B - Overview of Microscopy results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**48**	**96%**
	Gram-positive cocci	48	
0	**Incorrect gram stain and morphology results:**	**2**	**4%**
	Gram-negative bacilli	2	
NE	Not applicable	1	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Sample B - Overview of Final identification results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**45**	**90%**
	Staphylococcus aureus	44	
	Staphylococcus aureus-MRSA	1	
3	**Correct genus, no species specified:**	**1**	**2%**
	Staphylococcus species	1	
1	**Unnamed/unspecified micro-organism:**	**1**	**2%**
	a Gram-positive coccus	1	
0	**Misidentification of isolate:**	**3**	**6%**
	a Gram-negative bacillus	1	
	Escherichia coli	1	
	Escherichia coli O157	1	
NE	Not applicable	**1**	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Identification methods used included conventional methods (method based on cultivation procedures and manual biochemical identification), identification test kits e.g. Bacterial latex kits, chromogenic agar, automated methods and a combination of conventional methods with other methods. It is important to correlate results of basic biochemistry with the final identification of the organism irrespective of other methods used.

Table 6. Methods used for identification for Sample B with scores achieved

Methods used for organism identification	Number of participants	Scores achieved per method used			
		4	3	1	0
Bacterial latex antigen	1	1			
Chromogenic agar	1	1			
Conventional methods only	30	27	1	1	1
MALDI-TOF	4	4			
Molecular method with MALDI-TOF	1				1
Phoenix	3	3			
Vitek systems	5	4			1
Conventional methods with API	1	1			
Conventional methods with Biolog	1	1			
Conventional methods with Phoenix	1	1			
Conventional methods with Phoenix & MALDI-TOF	1	1			
Not stated	1	1			

Forty-five participants were assessed for antimicrobial susceptibility testing. Three participants misidentified the isolate, their AST results were not assessed and were automatically scored zero. One participant did not enter interpretations for the MIC results entered and one participant did not report on AST, both automatically scored zero. One participant provided a reason for not reporting AST results and was not evaluated. Participants were not penalised if they did not report results for all requested antimicrobial agents unless they had not reported a vital antibiotic (refer to scoring guide for more information).
Additional information for guidelines and test methods used by participants for AST are shown in table 7, an overview of Sample B AST results is shown in Figure 3.

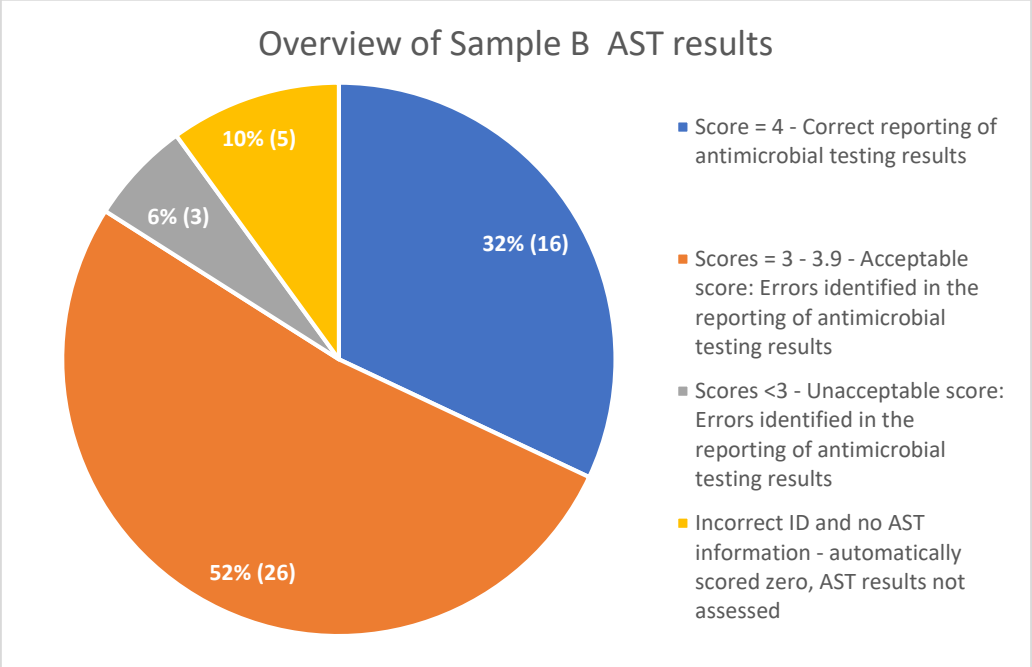


Figure 3. Overview of participant AST scores for Sample B. Not evaluated participants are excluded from the denominator when calculating percentages

Table 7. Guidelines and test methods used by participants for Sample B where AST results were assessed.

Criteria	Number of participants
Guidelines used:	
EUCAST	4
CA-SFM	6
CLSI	34
CLSI & EUCAST	1
AMR test method used:	
kirby-Bauer disk method	38
Kirby-Bauer disk method and a MIC method	2
MIC only	5
Oxacillin and or cefoxitin tested - to determine MRSA:	
Yes	35
No	10

Table 8. Summary of Antimicrobial susceptibility testing results for Sample B showing categorical agreement (NB: Excluding participants with misidentification of isolate and those who did not provide an interpretation for their entry)

Requested Antimicrobial agent	Participants performing Disk susceptibility testing						Participants performing MIC testing					
	Number of participants testing	R	I	S	%Acceptable	Acceptable response	Number of participants testing	R	I	S	%Acceptable	Acceptable response
Ampicillin	27	27	0	0	100	R	4	3	0	1	75	R
Cefoxitin	28	28	0	0	100	R	1	1	0	0	100	R
Chloramphenicol	24	2	2	20	83	S	1	0	0	1	100	S
Ciprofloxacin	31	30	1	0	97	R	3	3	0	0	100	R
Erythromycin	32	31	1	0	97	R	5	5	0	0	100	R
Clindamycin	23	3	2	18	78	S	5	0	0	5	100	S
Gentamicin	33	1	2	30	91	S	4	1	0	3	75	S
Linezolid	8	2	0	6	75	S	5	0	0	5	100	S
Oxacillin	15	14	0	1	93	R	5	5	0	0	100	R
Penicillin	20	20	0	0	100	R	5	5	0	0	100	R
Rifampicin	5	1	0	4	80	S	2	0	0	2	100	S
Quinupristin/Dalfopristin	6	0	2	4	67	S	0	0	0	0	0	S
Tetracycline	34	0	1	33	97	S	6	0	0	6	100	S
Trimethoprim/sulfamethoxazole	29	5	0	24	83	S	5	0	0	5	100	S
***Vancomycin**	11	4	1	6	N/A	N/A	7	0	0	7	100	S

*There are no disk susceptibility guidelines available for vancomycin. MIC tests must be used to determine vancomycin susceptibility results.

mE = Minor error
ME = Major error
VME = Very major error

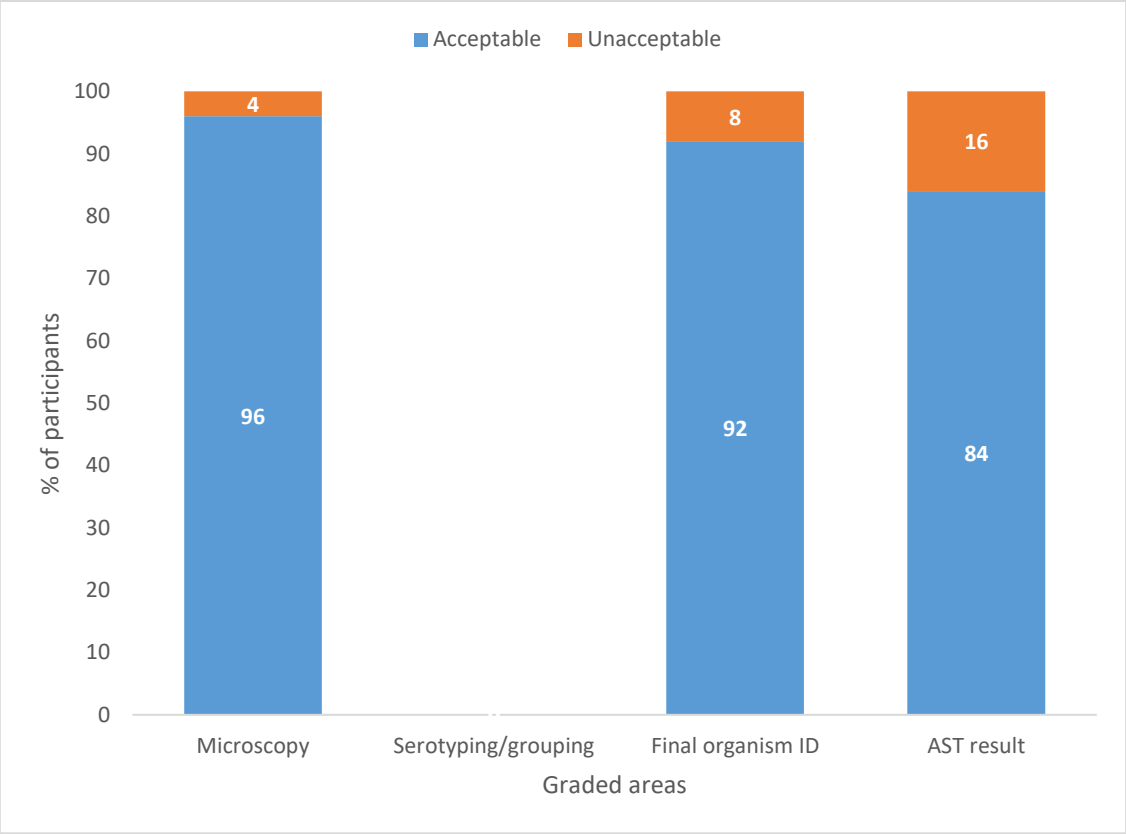


Figure 4. Summary of results for sample B shown as percentages of acceptable and unacceptable scores

Discussion

Staphylococcus species have a broad distribution in nature and consists of large populations. They are common commensals of the skin and mucous membranes of humans and animals and are ubiquitously recovered from the environment. Although during most of its existence they live as colonizers, when the skin and mucous membranes barrier of their host is impaired and the host is immunocompromised staphylococci may arise as important pathogens. Among all staphylococcal species, *S. aureus*, is considered to be the most pathogenic, being associated to a number of infections ranging from mild skin infections to life threatening diseases.[6]

The major driving force for the emergence of β-lactams resistance in staphylococci was the continuous exposure to β-lactams in multiple environments: in soils where they had to co-exist with penicillin-producing fungi; in production animal farms wherein large amounts of β-lactam antibiotics were used as food and during treatment of bacterial infections.[6]

It is vital to determine if a *S. aureus* isolated is methicillin susceptible or resistant. Testing for methicillin resistance can be performed using oxacillin or the surrogate agent cefoxitin. However, only cefoxitin is recommended for use with the disk diffusion method.[5] Participants not reporting either cefoxitin or oxacillin results were penalised for AST.

MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and –resistant isolates of other *Staphylococcus* species other than *S. aureus*, all of which give a similar sized zones of inhibition.[5]

Acceptable microscopy results were reported by 96% (n=48) of participants.

For identification of the organism for sample B, 92% (n=46) of participants reported acceptable responses. Of these, 2% (n=1) of participants correctly identified the organism to the genus level only and 90% (n=45) were able to correctly identify the organism to the species level i.e. *Staphylococcus aureus*. Misidentifications were reported by 8% (n=4) of participants.

Acceptable scores for antimicrobial susceptibility testing were achieved by 84% (n=42) of participants. Errors identified in AST results for participants reporting using disk susceptibility and MIC methods are shown in Table 8 above. Participants reporting vancomycin results using the disk susceptibility method were penalised as there are no guidelines available to interpret zone sizes. MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin.

Sample C

	Human health	Animal health
Clinical information	An organism isolated from urine specimen in sedated Intensive Care Unit patient.	A veterinarian observed in a poultry breeding farm birds with systemic infection, which manifested in diverse ways, including acute fatal septicaemia. Thus, high doses of ceftiofur was administered.
Sample type	Urine	Enlarged, hyperaemic liver of a chicken

Participants were requested to report on microscopy, identification and antimicrobial susceptibility testing for the pathogen isolated. The list of antimicrobial agents to be tested was provided. The sample contained an Extended Spectrum β-lactamase producing (ESBL) *Escherichia coli*. Participants performing serotyping on the *E. coli* isolated were not penalised when reporting the final identification. An overview of participant's performance is shown below. The isolate possess a number of resistance genes coding for phenotypic resistance, these are:

blaTEM-164/ blaTEM-206/ blaTEM-141/ blaTEM-34/ blaTEM-33, blaTEM-1B, blaCTX-M-15, blaOXA-181, sul2, drfA14/drfA1, tet(B), qnrS1

Sample C - Overview of Microscopy results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**45**	**94%**
	Gram-negative bacilli/rods	44	
	Gram-negative cocco-bacilli	1	
0	**Incorrect gram stain and morphology results:**	**3**	**6%**
	Gram-positive cocci	3	
NE	Not applicable	3	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Sample C - Overview of Final identification results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**42**	**86%**
	Escherichia coli	40	
	Escherichia coli - ESBL	1	
	Enteroinvasive *E. coli*	1	
3	**Correct genus, no species specified:**	**1**	**2%**
	Escherichia species	1	
1	***Escherichia* species other than coli / Unnamed/unspecified micro-organism:**	**1**	**2%**
	A Gram-negative bacillus	1	
0	**Misidentification of isolate:**	**5**	**10%**
	Salmonella enterica subsp *arizonae*	1	
	Salmonella Group A	1	
	Salmonella species - *Salmonella arizonae* (Enterosytem18R)	1	
	Staphylococcus aureus	2	
NE	Not applicable	**2**	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Identification methods used included conventional methods (method based on cultivation procedures and manual biochemical identification), identification test kits e.g. API, automated methods and a combination of conventional methods with other methods. It is important to correlate results of basic biochemistry with the final identification of the organism irrespective of other methods used. Oxidase and indole are tests that assist with the identification of isolates belonging to the *Enterobacterales* family and have been reported by a number of participants.

Table 9. Methods used for identification for Sample C with scores achieved

Methods used for organism identification	Number of participants	Scores achieved per method used			
		4	3	1	0
API	5	3			2
Conventional methods only	28	26	1	1	
MALDI-TOF	4	4			
Molecular methods and MALDI-TOF	1				1
Phoenix	2	1			1
Vitek systems	4	3			1
Conventional methods with API	1	1			
Conventional methods with Biolog	1	1			
Conventional methods with Phoenix	1	1			
Conventional methods with Phoenix & MALDI-TOF	1	1			
Not stated	1	1			

Forty-four participants were assessed for antimicrobial susceptibility testing. Five participants misidentified the isolate, their AST results were not assessed and were automatically scored zero. Two participants provided a reason for not reporting AST results and were not evaluated. Additional information for guidelines and test methods used by participants for AST are shown in table 10, an overview of Sample C AST results is shown in Figure 5. Although not evaluated, Extended spectrum β-lactamase (ESBL) results are shown in Table 10.

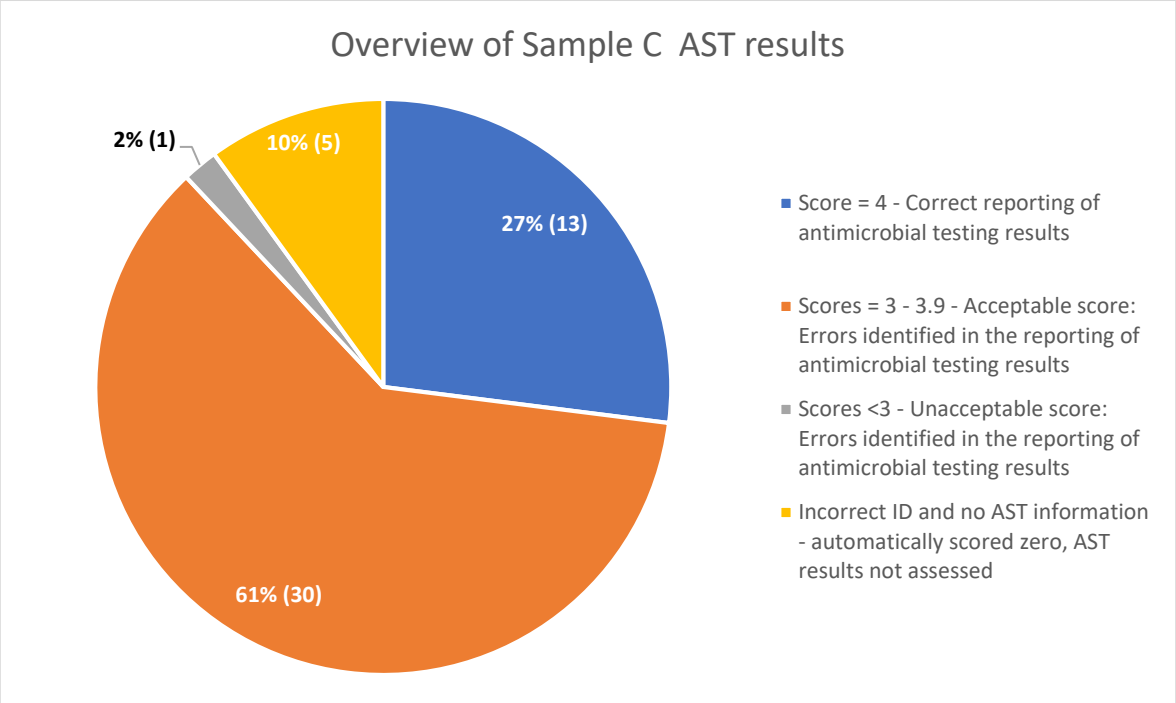


Figure 5. Overview of participant AST scores for Sample C. Not evaluated participants are excluded from the denominator when calculating percentages

Table 10. Guidelines and test methods used by participants for Sample C where AST results were assessed.

Criteria		Number of participants
Guidelines used:		
	EUCAST	3
	CA-SFM	6
	CLSI	34
	CLSI & EUCAST	1
AMR test method used:		
	kirby-Bauer disk method	36
	Kirby-Bauer disk method and a MIC method	3
	MIC only	5
ESBL reported		
	Positive	10
	Negative	1
	Undetermined	1

Table11. Summary of Antimicrobial susceptibility testing results for Sample C showing categorical agreement (NB: Excluding participants with misidentification of isolate and those who did not provide an interpretation for their entry)

Requested Antimicrobial agent	Participants performing Disk susceptibility testing						Participants performing MIC testing					
	Number of participants testing	R	I	S	%Acceptable	Acceptable response	Number of participants testing	R	I	S	%Acceptable	Acceptable response
Ampicillin	30	30	0	0	100	R	7	6	0	1	86	R
Amoxicillin-clavulanic acid	22	20	0	2	91	R	6	6	0	0	100	R
Cefepime	21	10	7	4	NE	NE	7	4	1	2	NE	NE
Cefotaxime	23	20	3	0	87	R	4	3	0	1	75	R
Cefoxitin	20	1	0	19	95	S	4	0	0	4	100	S
Ceftazidime	22	8	5	9	NE	NE	6	5	0	1	NE	NE
Ceftriaxone	31	30	0	1	97	R	5	5	0	0	100	R
Amikacin	21	1	2	18	86	S	8	0	0	8	100	S
Gentamicin	29	1	1	27	93	S	6	0	0	6	100	S
Tobramycin	10	2	0	8	80	S	2	0	0	2	100	S
Ertapenem	14	2	7	5	36	S	5	0	3	2	40	S
Imipenem	15	3	4	8	53	S	3	0	2	1	33	S
Meropenem	26	3	7	16	62	S	6	0	0	6	100	S
Nitrofurantoin	19	0	1	18	95	S	6	0	0	6	100	S
Trimethoprim/sulfamethoxazole	26	24	1	1	92	R	7	7	0	0	100	R

mE = Minor error
ME = Major error
VME = Very major error

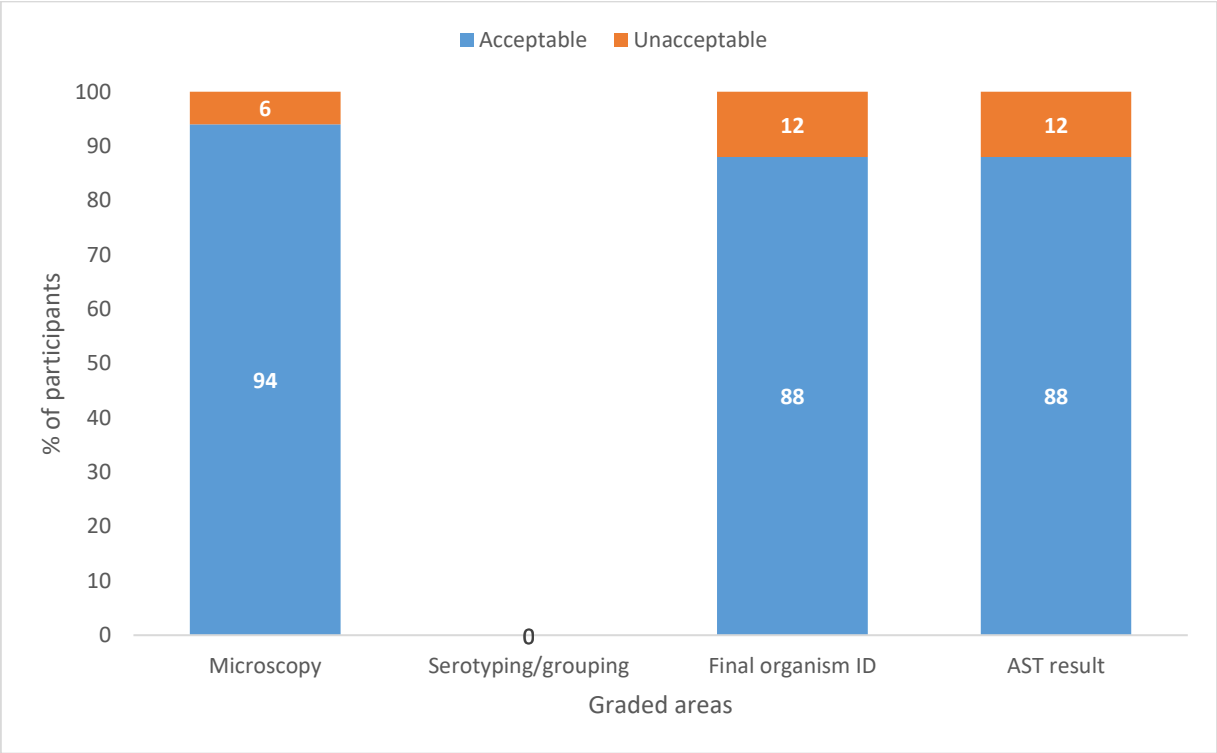


Figure 6. Summary of results for sample C shown as percentages of acceptable and unacceptable scores

Discussion

Acceptable microscopy results were reported by 94% (n=45) of participants.

For identification of the organism for sample C, 88% (n=43) of participants reported acceptable responses. Of these, 2% (n=1) of participants correctly identified the organism to the genus level only and 86% (n=45) were able to correctly identify the organism to the species level i.e. *Escherichia coli.* Misidentifications were reported by 10% (n=5) of participants.

Acceptable scores for antimicrobial susceptibility testing were achieved by 88% (n=43) of participants. Errors identified in AST results for participants reporting using disk susceptibility and MIC methods are shown in Table 11 above. When using the current breakpoints, routine ESBL testing is no longer necessary before reporting results (i.e. it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillin's to resistant). However, ESBL testing may still be useful for epidemiological or infection prevention purposes.[5]

Sample D

	Human health	Animal health
Clinical information	A patient presented to casualty with abdominal cramps, diarrhoea, fever and chills.	A veterinarian was called to investigate lack of feed consumption, diarrhoea and a decrease in egg production in a layer farm.
Sample type	Blood culture	A colonized caeca from chicken

Participants were requested to report on microscopy, serotyping/serogrouping (if applicable), identification and antimicrobial susceptibility testing for the pathogen isolated. The list of antimicrobial agents to be tested was provided. The sample contained a *Salmonella* Paratyphi B non-susceptible to ciprofloxacin possessing the *gyrA (D87G)* gene as well as *aac(6')-Iaa* resistance gene. An overview of participant's performance is shown below.

Sample D - Overview of Microscopy results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**47**	**96%**
	Gram-negative bacilli	45	
	Gram-negative cocco-bacilli	2	
1	**Partially correct result:**	**1**	**2%**
	Gram-positive cocci and Gram-negative bacilli	1	
0	**Incorrect gram stain and morphology results:**	**1**	**2%**
	Gram-positive Yeast	1	
NE	Not applicable	2	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Sample D - Overview of serotyping results

Score	Response	Number of participants	Percentage
4	**Acceptable response:**	**2**	**12,5%**
	Salmonella group O:4 (B)	2	
3	**Partially correct:**	**8**	**50%**
	Polyvalent positive	2	
	Salmonella polyvalent positive	4	
	Salmonella polyvalent positive - Antiserum OMA POSITIF	1	
	Salmonella polyvalent positive - Salmonella O and H were both Positive	1	
1	**Incorrect serotype specified :**	**4**	**25%**
	Salmonella Enteritidis O:9 (D)	1	
	Salmonella Typhi O:2 (A)	1	
	Salmonella Typhimurium O:4 (B) - OMA Positive, O:4,5 Positive	1	
	Salmonella Typhimurium O:4 (B) - Poly O-Positive,09-NEGATIVE	1	
0	**Incorrect result OR Not reported:**	**2**	**12,5%**
	Enterohaemorrhagic *E.coli* (EHEC)	1	
	Pseudomonas aeruginosa	1	
NE*	**Reasons provided for not reporting serotyping/serogrouping results:**	**35**	
	Reagent or consumables unavailable	27	
	Not done	1	
	Not applicable	5	
	Instrument out of service	1	
	Other	1	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Sample D - Overview of Final identification results			
Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**36**	**74%**
	Salmonella Group B	2	
	Acceptable responses for participants reporting partially correct serotype results or those not evaluated for serotyping:		
	Salmonella (non-typeable)	1	
	Salmonella (non-Typhi)	2	
	Salmonella (non-Typhi) - No *Salmonella Typhi*, No *S*. ParatyphIi A	1	
	Salmonella enterica	2	
	Salmonella enterica subsp *enterica*	2	
	Salmonella group	7	
	Salmonella Paratyphi B	1	
	Salmonella species	17	
	a Gram-negative bacillus - Production of H2S suggests *Salmonella* species	1	
3	***Salmonella* with species not specified:**	**1**	**2%**
	Salmonella species	1	
1	**Other *Salmonella* species:**	**8**	**16%**
	Salmonella Typhi	1	
	Salmonella Typhimurium	5	
	Staphylococcus aureus - query *salmonella*	1	
	a Gram-negative bacillus	1	
0	**Misidentification of isolate:**	**4**	**8%**
	Citrobacter species	1	
	Enteropathogenic *E. coli* (Pool C)	1	
	Pseudomonas aeruginosa	1	
	Pseudomonas aeruginosa (mucoid)	1	
NE	Not applicable	**2**	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Identification methods used included conventional methods (method based on cultivation procedures and manual biochemical identification), identification test kits e.g. API, automated methods and a combination of conventional methods with other methods. Participants who were unable to perform serotyping or those who reported partially correct serotyping results were not penalised for not reporting the genus and species of the isolate.

Table 12. Methods used for identification for Sample D with scores achieved

Methods used for organism identification	Number of participants	Scores achieved per method used			
		4	3	1	0
API	6	5		1	
Conventional methods only	26	19		4	3
MALDI-TOF	4	4			
Molecular, MALDI-TOF and chromogenic agar	1				1
Phoenix	1	1			
Vitek systems	5	3		2	
Conventional methods with API	2	2			
Conventional methods with Biolog	1	1			
Conventional methods with Phoenix	2	1		1	
Conventional methods with Phoenix & MALDI-TOF	1	1			

Forty-three participants were assessed for antimicrobial susceptibility testing. Five participants misidentified the isolate, their AST results were not assessed and were automatically scored zero. One participant did not enter interpretations for the results entered and automatically scored zero. Two participants provided a reason for not reporting AST results and were not evaluated. Additional information for guidelines and test methods used by participants for AST are shown in Table 13, an overview of Sample D AST results is shown in Figure 7.

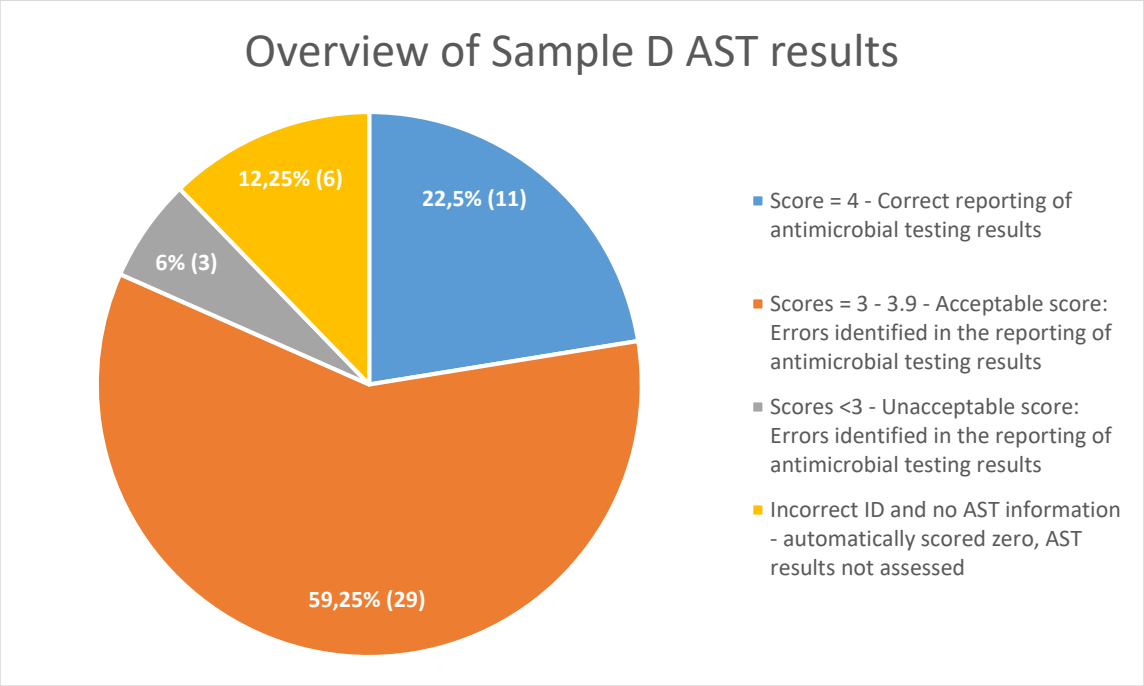


Figure 7. Overview of participant AST scores for Sample D. Not evaluated participants are excluded from the denominator when calculating percentages

Table 13. Guidelines and test methods used by participants for Sample D where AST results were assessed.

Criteria	Number of participants
Guidelines used:	
EUCAST	4
CA-SFM	6
CLSI	30
CLSI & EUCAST	1
Unknown guideline	1
AMR test method used:	
kirby-Bauer disk method	35
Kirby-Bauer disk method and a MIC method	2
MIC only	6

Table 14. Summary of Antimicrobial susceptibility testing results for Sample D showing categorical agreement (NB: Excluding participants with misidentification of isolate and those who did not provide an interpretation for their entry)

Requested Antimicrobial agent	Participants performing Disk susceptibility testing						Participants performing MIC testing					
	Number of participants testing	R	I	S	%Acceptable	Acceptable response	Number of participants testing	R	I	S	%Acceptable	Acceptable response
Ampicillin	30	4	2	24	80	S	7	0	0	7	100	S
Amoxicillin-clavulanic acid	26	1	1	24	92	S	5	0	0	5	100	S
Cefepime	20	1	1	18	90	S	7	0	0	7	100	S
Cefotaxime	21	2	0	19	90	S	4	0	0	4	100	S
Cefoxitin	23	0	0	23	100	S	3	2	0	1	33	S
Ceftazidime	27	2	1	24	89	S	6	0	0	6	100	S
Amikacin	24	0	1	23	96	S	6	3	0	3	50	S
Gentamicin	28	0	3	25	89	S	5	3	0	2	40	S
Tobramycin	11	1	0	10	91	S	2	1	0	1	50	S
*Ciprofloxacin	20	1	7	12	35	I*	4	0	0	4	0	I
Ertapenem	16	0	0	16	100	S	5	0	0	5	100	S
Imipenem	18	0	0	18	100	S	3	0	0	3	100	S
Meropenem	27	1	1	25	93	S	5	0	0	5	100	S
Trimethoprim/sulfamethoxazole	28	2	0	26	93	S	6	0	0	6	100	S

* There are no EUCAST disk susceptibility guidelines for ciprofloxacin. Numbers reported for ciprofloxacin disk susceptibility testing are only for participants using CLSI guidelines.

mE = Minor error

ME = Major error

VME = Very major error

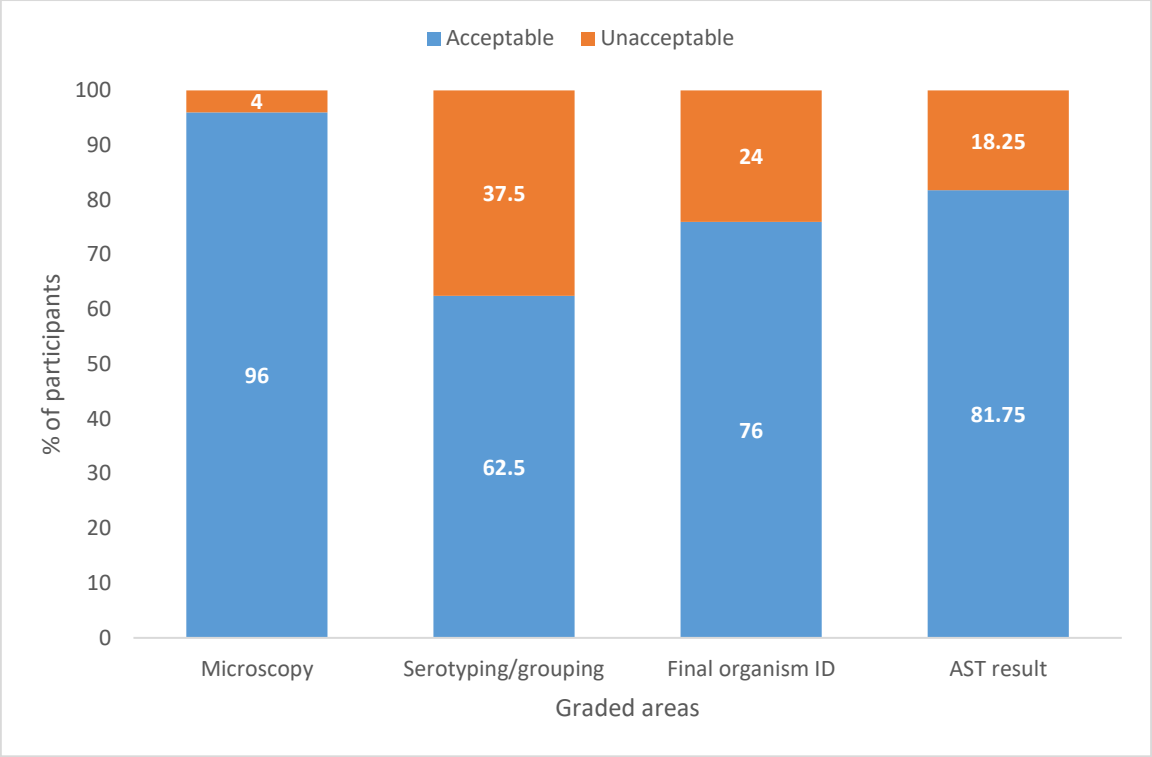


Figure 8. Summary of results for sample D shown as percentages of acceptable and unacceptable scores

Discussion

Acceptable microscopy results were reported by 96% (n=47) of participants.

Serotyping/serogrouping was not evaluated for 35 participants. Acceptable responses were received from 62.5% (n=10) of participants. Of these 12.5% (n=2) of participants correctly reported *Salmonella* group O:4 (B) and 50% (n=8) reported partially correct serotyping/serogrouping results.

For identification of the organism for sample D, participants who were unable to perform serotyping or those who reported partially correct serotyping results were not penalised for not reporting the genus and species of the isolate, 76% (n=37) of participants reported acceptable responses. Salmonella with an incorrect species was reported by 16% (n-8) of participants. Misidentifications were reported by 8% (n=4) of participants.

Acceptable scores for antimicrobial susceptibility testing were achieved by 81.75% (n=40) of participants. Errors identified in AST results for participants reporting using disk susceptibility and MIC methods are shown in Table 14 above. There are no EUCAST disk susceptibility guidelines for ciprofloxacin, participants using EUCAST guidelines were penalised when providing ciprofloxacin results using disk susceptibility. It was observed that laboratories that tested ciprofloxacin, reported the correct expected zone size or MIC but a large number of participants interpreted these incorrectly. Participants should be aware that there are different zone sizes and breakpoints (irrespective of the guidelines used) for *Salmonella* species versus other *Enterobacterales*.

As per EUCAST guidelines, susceptibility of *Salmonella* species to ciprofloxacin can be inferred from perfloxacin disk diffusion susceptibility. The 5µg perfloxacin disk is used as a screening method to detect ciprofloxacin resistance in *Salmonella*.[7]

Sample E

	Human health	Animal health
Clinical information	After eating raw oysters on the previous evening, a patient presents with watery diarrhoea, abdominal cramps and vomiting.	A fish farm called a veterinarian to examine farmed Tilapia due to extensive problems with tail and fin rot.
Sample type	Stool	Tissue and tail parts from Tilapia nilotica

Participants were requested to report on microscopy, serotyping/serogrouping (if applicable) and identification. The sample contained *Vibrio parahaemolyticus.* An overview of participant's performance is show below.

Sample E - Overview of Microscopy results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**46**	**96%**
	Gram-negative bacilli or curved bacilli	45	
	Gram-negative cocco-bacilli	1	
0	**Incorrect gram stain and morphology results:**	**1**	**2%**
	Yeast	1	
	No growth	**1**	**2%**
NE	Not applicable	**3**	

Sample E - Overview of Final identification results

Score	Response	Number of participants	Percentage
4	**Acceptable responses:**	**23**	**48%**
	Vibrio parahaemolyticus	19	
	Vibrio parahaemolyticus/ alginolyticus	4	
3	**Correct genus, no species specified:**	**2**	**4%**
	Vibrio species	2	
1	***Vibrio* species other than parahaemolyticus / Unnamed/unspecified microrganism:**	**7**	**15%**
	V.cholerae O1 serotype Inaba	1	
	Vibrio cholerae non-O1	1	
	Vibrio furnissii	1	
	Vibrio metschnikovii	1	
	Vibrio vulnificus	1	
	a Gram negative bacillus	2	
0	**Misidentification of isolate:**	**16**	**31%**
	Aeromonas salmonicida	1	
	Aeromonas species	2	
	Candida haemulonii var. *vulnera*	1	
	Escherichia coli	1	
	Pseudomonas aeruginosa	1	
	Pseudomonas species	2	
	Pseudomonas species - *Staphylococcus aureus* also isolated	1	
	Salmonella species	1	
	Shigella group - MIXED WITH PROTEUS	1	
	Shigella species	2	
	a non-fermenting Gram negative bacillus	2	
	No growth	**1**	**2%**
NE	Not applicable	**3**	

*NE – Not evaluated (these numbers are excluded from the denominator when calculating percentages)

Identification methods used included conventional methods (method based on cultivation procedures and manual biochemical identification), identification test kits e.g. API, automated methods, molecular methods and a combination of conventional methods with other methods. It is important to correlate results of basic biochemistry with the final identification of the organism irrespective of other methods used. Serotyping was not evaluated.

Table 15. Methods used for identification for Sample E with scores achieved

Methods used for organism identification	Number of participants	Scores achieved per method used			
		4	3	1	0
API	5	3		2	
Conventional methods only	25	10		3	12
MALDI-TOF	4	4			
Molecular methods and MALDI-TOF	1				1
Phoenix	2	1			1
Vitek systems	3	3			
Conventional methods with API	2		1	1	
Conventional methods with Biolog	1			1	
Conventional methods with Phoenix	1				1
Conventional methods with MALDI-TOF	1	1			
Conventional methods with molecular methods	1		1		
Not stated	1	1			

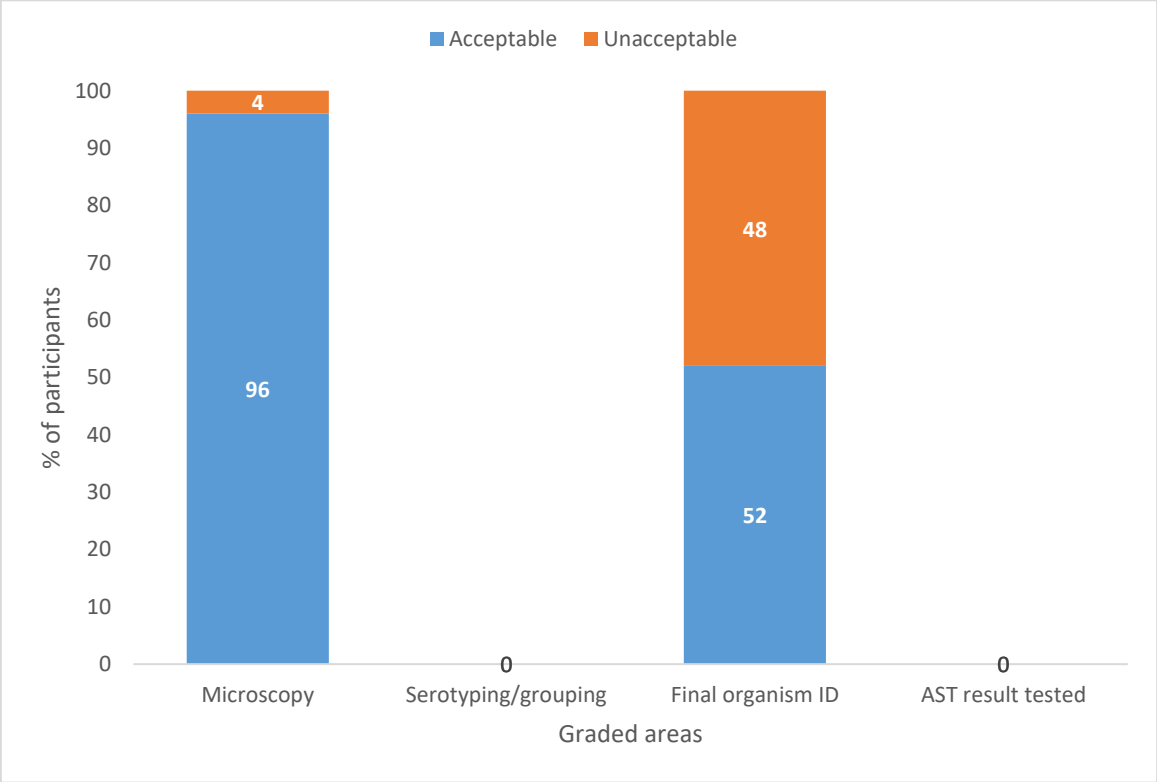


Figure 9. Summary of results for sample E shown as percentages of acceptable and unacceptable scores

Discussion

Vibrio spp. are primarily found in aquatic habitats. Their distribution and abundance depends on water temperature, sodium concentration, nutrient content, and the presence of certain plant and animal species. Species that require only low sodium concentrations (e.g. *V. cholerae*, *V. mimicus*) can be found in freshwater. *V. parahaemolyticus* is frequently found in codfish, sardine, mackerel, flounder, clam, octopus, shrimp, crab, lobster, crawfish, scallop, and oyster.

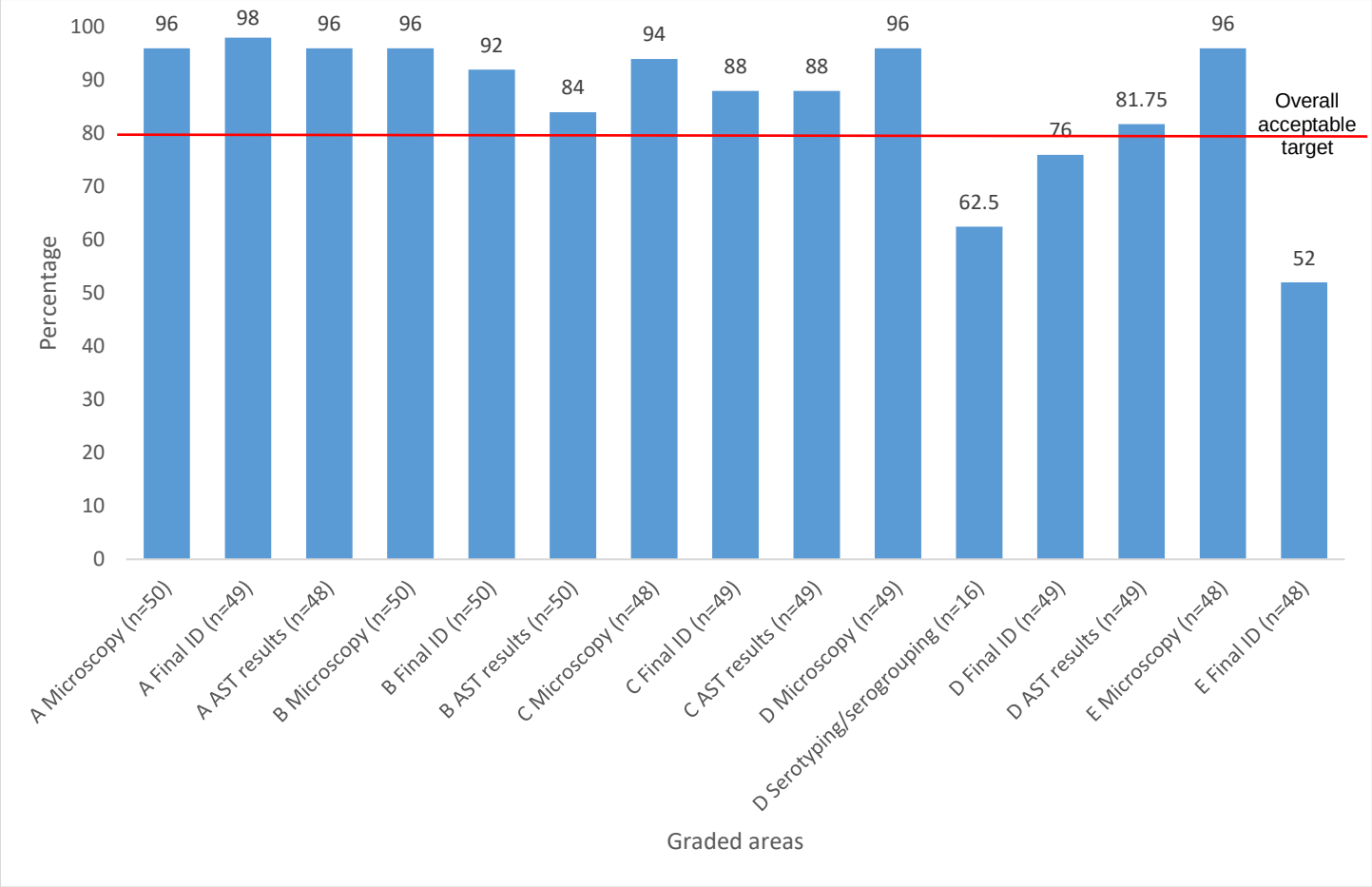
Acceptable microscopy results were reported by 96% (n=46) of participants.

For identification of the organism for sample E, 52% (n=45) of participants reported acceptable responses. Of these, 4% (n=2) of participants correctly identified the organism to the genus level only and 48% (n=23) were able to correctly identify the organism to the species level i.e. *Vibrio parahaemolyticus.* Misidentifications were reported by 31% (n=16) of participants.

General comments

Sixty-seven participants were enrolled to participate in this pilot of the EQuAfrica programme. Responses were received from 51 participants, one of which submitted no results for all samples. It is advisable to indicate inability to participate in a cycle when completing pre-reporting questions.

Figure 10. Percentage of acceptable (scores 3 or 4) results for all grading areas across all samples.



n= number of participants evaluated per graded area

The overall acceptable target of 80% was met for all samples where microscopy was evaluated.

Identification of the organism in Sample A(96%), B(92%) and C (88%) were well done, with participants achieving an acceptable response of over 80%. Identification of the organism in sample D (76%) and E (52%) did not meet the overall acceptable target of 80%.

For guidelines and additional information regarding the identification of *Vibrio* species please see the information in the guidance documents in the following link:
https://www.gov.uk/government/publications/smi-id-19-identification-of-vibrio-species

Serotyping/serogrouping was applicable for sample D only. Of the participants submitting responses, 62.5% achieved acceptable results and did not meet the overall acceptable criteria of 80%.

For additional information regarding the identification of *Salmonella* species, please refer to the guidance documents in the link below:
UK SMI ID 24: identification of Salmonella species (publishing.service.gov.uk)

Antimicrobial susceptibility testing was requested for samples A, B, C and D. Participants were provided with a list of antibiotics to be tested. The overall acceptable target of 80% was achieved for all samples where AST results were evaluated. Participants must follow CLSI or EUCAST/CA-SFM guidelines to the smallest detail, there is no partial adherence to guidelines.
For troubleshooting and corrective actions for AST, pay attention to the variables that must be controlled in the performance of routine AST and MIC test methods, specifically:

- Inoculum – use the correct turbidity of 0.5McFarland.
- Use the correct media as per guideline in use.
- Cation concentration and pH – if not controlled can lead to detection of false resistance or susceptibility for specific antibiotics.

- Agar depth – possibility for false susceptibility if <3mm and false resistance if >5mm due to diffusion of antibiotic agent into the media.
- Incubation atmosphere – ensure the correct atmospheric conditions are used for the organism under test as stated in the guidelines in place in the laboratory..
- Temperature and duration of incubation – incubate prepared agar plates at the correct temperature for the organism type. Some antibiotic/organism combinations require a full 24hr incubation, e.g. vancomycin resistant enterococci may go undetected if <24 hours incubation.
- Antimicrobial disks – use disks with proper FDA/CLSI-defined concentration of drug.
  Ensure disks are stored correctly.
  Make sure disks used are not expired and pass quality control when used.
  Proper placement of disks on agar to avoid overlapping zones.
- Solutions – make sure prepared from reference standard powders.
- End-point measurement – read disk susceptibility profiles according to the correct method specified in guidelines used. E.g. the correct use of reflected or transmitted when reading zone sizes, use of adequate light and reading devices when reading MICs.

In general, ensure that samples are correctly labelled when culturing, work aseptically to avoid contamination of the sample. Ensure correct result entry to avoid penalties when graded.

Participants are encouraged to contact the EQA provider if they encounter any problems: equafricapt@aslm.org

Thank you to all laboratories participating in the EQuAFrica pilot programme.

Thank you to all referee laboratories for their continual support.

Comments prepared by NICD and reviewed by members of the EQuAfrica programme advisory committee.

* * End of Report **

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