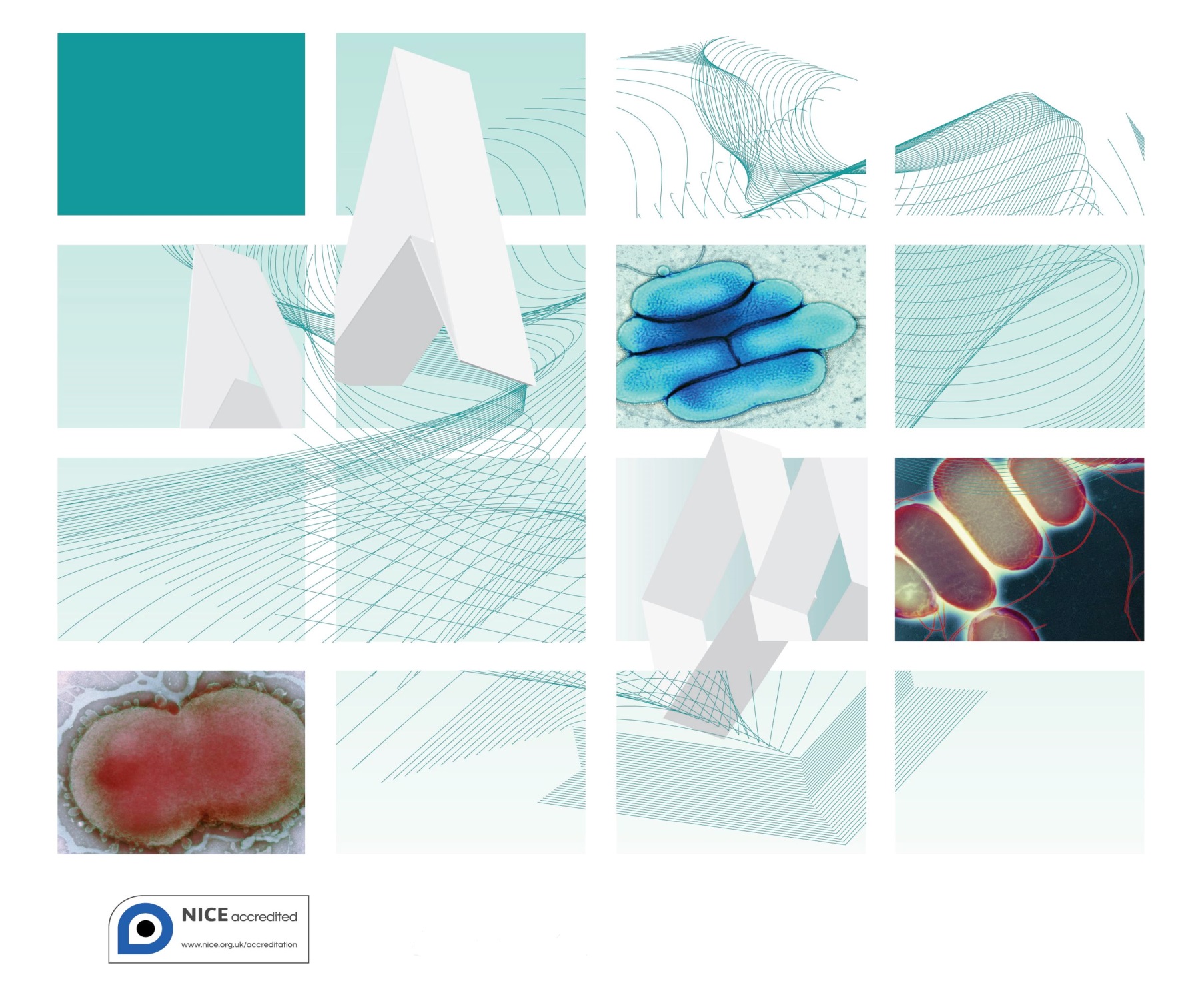
UK Standards for Microbiology Investigations

Identification of *Enterobacteriaceae*



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

For further information please contact us at:

Standards Unit

Microbiology Services

Public Health England

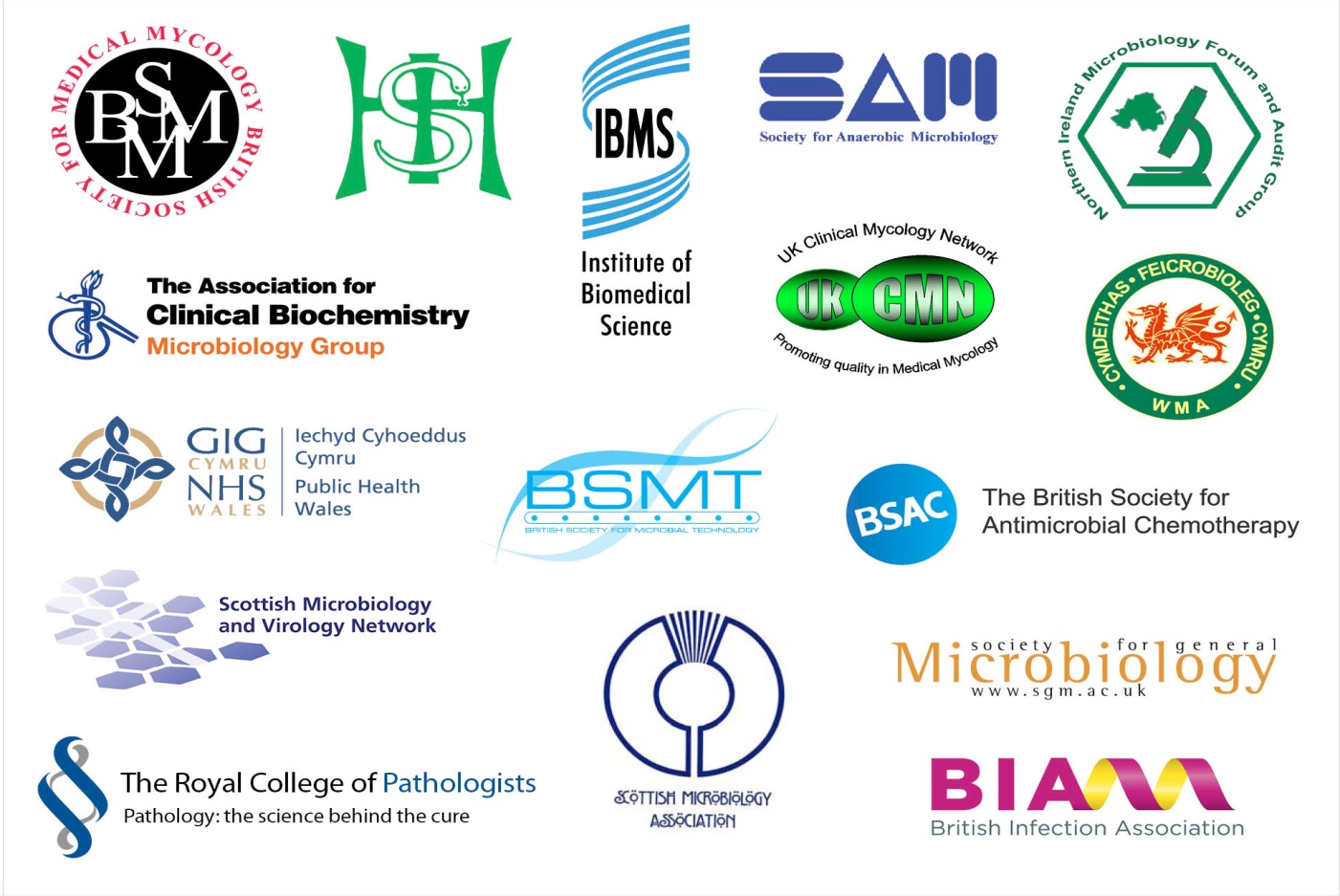
61 Colindale Avenue

London NW9 5EQ

E-mail: [standards@phe.gov.uk](mailto:standards@phe.gov.uk)

Website: <http://www.hpa.org.uk/SMI>

UK Standards for Microbiology Investigations are produced in association with:



Contents

Acknowledgments 2

Amendment Table 4

UK Standards for Microbiology Investigations: Scope and Purpose 5

Scope of Document 8

Introduction 8

Technical Information/Limitations 13

1 Safety Considerations 14

2 Target Organisms 16

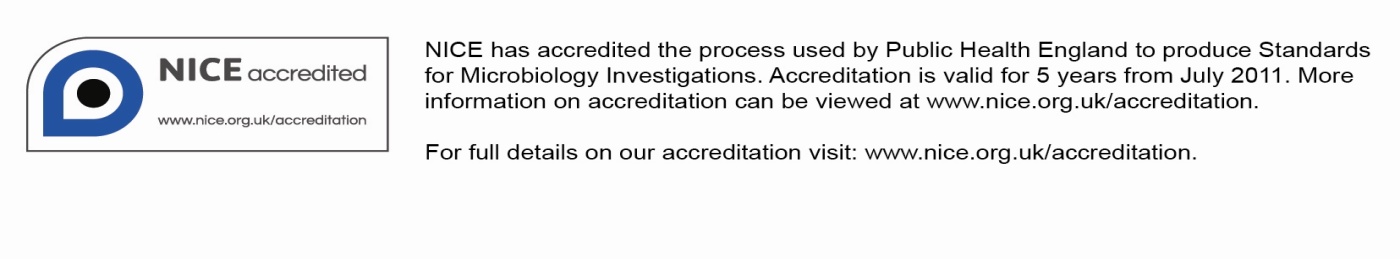
3 Identification 17

4 Identification of *Enterobacteriaceae* Flowchart 23

5 Reporting 24

6 Referrals 26

References 27



Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

|  |  |
| --- | --- |
| Amendment No/Date. | 5/ |
| Issue no. discarded. | 3.1 |
| Insert Issue no. | dp+ |
| **Section(s) involved** | **Amendment** |
|  |  |

|  |  |
| --- | --- |
| Amendment No/Date. | 4/21.10.11 |
| Issue no. discarded. | 3 |
| Insert Issue no. | 3.1 |
| **Section(s) involved** | **Amendment** |
| Whole document.. | Document presented in a new format. |
| References. | Some references updated. |

UK Standards for Microbiology Investigations[[1]](#footnote-1)#: Scope and Purpose

Users of SMIs

* SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK
* SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests
* SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives <http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313>. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (). Identification of *Enterobacteriaceae*. UK Standards for Microbiology Investigations. ID 16 Issue dp+. <http://www.hpa.org.uk/SMI/pdf>.

Scope of Document

This SMI describes the identification of members of the family *Enterobacteriaceae.* There are a large number of species included in the family. In diagnostic clinical microbiology laboratories, it is usual to attempt identification by use of biochemical tests. The level of identification depends on the site of infection, the immune status of the host and the need for epidemiological surveillance.

Because of the large number of species involved, this SMI will concentrate on the most common genera and species isolated from clinical specimens. The identification of *Enterobacteriaceae* can be simplified by taking advantage of the fact that three species comprise 80-95% of all isolates in the clinical setting. These are *Esherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis1*. The other species can be easily identified using biochemical tests.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

Taxonomically, the bacterial family *Enterobacteriaceae* currently has 53 genera (and over 170 named species) and they include *Arsenophonus, Biostraticola, Brenneria, Buchnera, Budvicia, Buttiauxella, Calymmatobacterium, Cedecea, Citrobacter, Cosenzaea, Cronobacter, Dickeya, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Gibbsiella, Hafnia, Klebsiella, Kluyvera, Leclercia, Leminorella, Levinea, Lonsdalea, Mangrovibacter, Moellerella, Morganella, Obesumbacterium, Pantoea, Pectobacterium, Phaseolibacter, Photorhabdus, Plesiomonas, Pragia, Proteus, Providencia, Rahnella, Raoultella, Saccharobacter, Salmonella, Samsonia, Serratia, Shigella, Shimwellia, Sodalis, Tatumella, Thorsellia, Trabulsiella, Wigglesworthia, Xenorhabdus, Yersinia and Yokenella.* Of these, 26 genera are known to be associated with infections in humans.

The nomenclature of the *Enterobacteriaceae* is complicated and has been based on biochemical and antigenic characteristics. Recently, the application of new technologies such as DNA hybridisation has resulted in numerous changes in classification of the *Enterobacteriaceae*1. Many new genera and species have been discovered, some unusual and rare, and many species have also been reclassified to other genera eg the transfer of *Enterobacter sakazakii* to the *Cronobacter sakazakii*2.

Characteristics

Members of the *Enterobacteriaceae* are small Gram negative, non-sporing straight rods. Some genera are motile by means of peritrichous flagella except *Tatumella*, *Shigella* and *Klebsiella* species which are non-motile. They are facultatively anaerobic and most species grow well at 37°C, although some species grow better at 25-30°C. They grow well on peptone and meat extract media. Some strains grow on D- glucose as the sole source of carbon and energy, but other strains require vitamins and or amino acids. Acid is produced during the fermentation of D- glucose and other carbohydrates3,4.

They are oxidase negative and catalase reactions vary among *Enterobacteriaceae*. Nitrates are reduced to nitrites except by some strains of *Erwinia.* They are distributed worldwide and may be found in soil, water, plants, humans and animals.

Medically important genera of the family *Enterobacteriaceae* are;

*Citrobacter* species

There are 11 species of which 10 have been recovered from clinical material5. They may be found in the faeces of humans and animals as part of the normal flora and grow readily on ordinary media.

Cells are short rods arranged singly, in pairs, or in short chains with rounded ends. They are motile with pertrichous flagella. Colonies are generally grey, smooth and moist although mucoid or rough strains occur. Some strains of *Citrobacter* resemble *Salmonella* species biochemically and agglutinate with *Salmonella* polyvalent antisera, which may lead to misidentification6.

They are positive for indole, catalase and nitrate reduction tests. Acid and gas is produced from aesculin, arabinose, glucose, galactose, glycerol, inositol, lactose, levulose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose, and xylose.

*Enterobacter* species

There are 26 species and 2 subspecies, of which recently, 6 has been reclassified to other genera. Only 10 have been isolated from clinical material7. They grow readily on ordinary media, ferment glucose with the production of acid and gas, and are motile by peritrichous flagella. Some strains with a K antigen possess a capsule. Colonies of *Enterobacter* strains may be slightly mucoid. They are catalase positive and oxidase negative. Nitrates are also reduced. They also ferment glucose and lactose with the production of acid and gas.

*Enterobacter* has the general characteristics of *Klebsiella* species but can be differentiated because they are motile and ornithine positive.

*Enterobacter* species are widely distributed in nature. They are found in the soil, water, dairy products, and in the intestines of animals as well as humans.

*Escherichia* species

There are 5 species and all are known to cause human disease8. Cells are typically rod-shaped, non-spore forming, motile with peritrichous flagella or non-motile, and are about 2.0μm long and 0.25 - 1.0μm in diameter. They are able to grow under aerobic and anaerobic conditions. Optimal growth occurs at 37°C. On MacConkey agar, colonies are either red or colourless and about 2 - 3mm in diameter. They are catalase positive and oxidase negative. Nitrates are also reduced.

The most commonly isolated is *Escherichia coli*, which contains numerous serotypes, some of which are associated with specific diseases. A number of strains of *E. coli* may produce enterotoxins or other virulence factors, including those associated with invasiveness. Some strains are capsulated with a K antigen.

For more information on the identification of E. coli O157, refer to [ID 22 - Identification of *Escherichia coli* O157](http://www.hpa.org.uk/SMI/pdf/Identification).

*Hafnia* species

The genus *Hafnia* currently has 2 species9. The optimum temperature for growth is 35°C. It grows readily on ordinary media and is generally motile. Motility is more pronounced at 30°C than 37°C 10. *H. alvei* can resemble non-motile *Salmonella* biochemically, and can agglutinate in polyvalent salmonella antisera.

On moderately selective agars, they typically appear as large, smooth, convex, translucent colonies of 2 to 3 mm in diameter with an entire edge; some may exhibit an irregular border. Some strains of *Hafnia* also produce red or pink colonies on xylose-lysine-desoxycholate agar.

They are oxidase and indole negative and are positive for nitrate reduction and for enterobacterial common antigen. They also produce acid with or without gas from the metabolism of D-glucose and other carbohydrate or carbohydrate-like compounds 11.

*Klebsiella* species

The genus *Klebsiella* contains 6 species and 3 subspecies. There are 4 species related to humans and they include *K. pneumoniae subspecies pneumoniae, ozaenae,* and *rhinoscleromatis; K. oxytoca; K. granulomatis and K. variicola.* The other two species*, K. singaporensis* and *K. michiganensis* have been isolated from the soil and from a tooth brush holder respectively. The genus consists of over 77 capsular antigens (K antigens), leading to different serogroups12. These well-developed polysaccharide capsules give the colonies their characteristic mucoid appearance.

*Klebsiella* species are non-motile, usually encapsulated rod-shaped bacteria, belonging to the family *Enterobacteriaceae*. These bacteria produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test as well as indole and urease tests. They are generally facultatively anaerobic, and range from 0.3 to 1.0mm in width and 0.6 to 6.0mm in length13.

All strains grow readily on ordinary media. On MacConkey agar, the colonies typically appear large, mucoid, and red, with red pigment usually diffusing into the surrounding agar, indicating fermentation of lactose and acid production.

They can cause bacteraemia and hepatic infections, and have been isolated from a number of unusual infections, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis14.

*Morganella* species

The genus *Morganella* contains 2 species, and only one is known to cause infections in humans, *Morganella morganii*15. *M. morganii* is divided into 2 sub species on the basis of their abilities to ferment trehalose. On nutrient agar, colonies are 1 to 2mm in diameter, greyish, and opaque, circular, convex, and smooth with entire edges after 24 h at 35°C. Good growth also occurs at 22°C.

They are motile with peritrichous flagella, but some strains do not form flagella above 30°C. *M. morganii* can resemble non-motile *Salmonella* biochemically, and can agglutinate in polyvalent salmonella antisera. They are urease and indole positive as well as oxidase negative. Acid and gas are produced from utilizing glucose. Acid is also produced from mannose, galactose and trehalose16.

They have been isolated from human clinical specimens (stool, wound, sputum, eye, bile, gastric ulcer, urine)16.

*Plesiomonas* shigelloides

The genus *Plesiomonas* contains one specie, *Plesiomonas shigelloides*17. Cells are short gram-negative rods. They are generally 0.3-1.0 µm in width, 0.6-6.0µm in length, motile with lophotrichous polar flagella, non-spore-producing, and facultatively anaerobic. They are able to grow at salt concentrations of 0-5%, at a pH of 4.0-8.0, and at temperatures of 8 - 44°C.

*Plesiomonas shigelloides* will grow on most enteric media where they produce non-lactose, non-sucrose fermenting colonies. On Inositol Brilliant Green Bile Agar, they have pink colonies and on blood agar, colonies are 2-3 mm in diameter, large grey, opaque, and convex, β-haemolytic colonies after incubation at 35-37°C for 16-24 hours.

They are also oxidase-positive and catalase-positive and can be distinguished from other *Enterobacteriaceae* as it is the only oxidase positive genus in this family.

*Plesiomonas* is negative for DNAse; this and other biochemical tests (Moeller’s lysine, ornithine, and arginine tests and fermentation of meso-inositol) distinguish it from *Aeromonas* species.

Some *Plesiomonas* strains share antigens with *Shigella sonnei*, and cross-reactions with *Shigella* antisera occur 18.

The have been isolated from human clinical specimens - faeces, blood, CSF, wounds, respiratory tract and urine. It has also been isolated from fresh water, freshwater fish, and shellfish and from many types of animals19.

*Proteus* species

There are 4 species of *Proteus*, of which 3 cause disease (see section 2)20. Since it belongs to the family of *Enterobacteriaceae*, general characteristics are applied on this genus. All strains are motile. They may swarm on blood agar, producing concentric zones or an even film. On MacConkey agar, colonies are colourless, flat, often swarm slightly and are 2-3mm in diameter (this is specific to *Proteus vulgaris* and *Proteus mirabilis*). Other species do not swarm. They are resistant to polymyxin B and colistin. Proteus species can resemble non- motile *Salmonella* biochemically, and can agglutinate in polyvalent salmonella antisera.

*Proteus* species do not ferment lactose, but have shown to be capable lactose fermenters depending on the species in a triple sugar iron (TSI) test. They are also oxidase negative but catalase and nitrate positive. Other specific tests are the urease (which is an essential test to differentiate *Proteus* from *Salmonella*) and phenylalanine deaminase tests. All strains are urease positive.

They have been isolated from human faeces, urine, abdominal, neck, groin, and hip wounds, infected conjunctiva, sacral decubitus, and sputum21.

*Providencia* species

The genus *Providencia* was originally established for organisms similar to *Proteus* species that were urease negative. There are 8 species within the genus, of which 3 cause disease (see section 2)22. All species are motile. On blood agar and MacConkey agar, colonies are colourless, flat, 2-3mm in diameter and do not swarm.

They do not ferment lactose and little gas is produced from fermentable carbohydrates. They do not produce hydrogen sulphide and urea is not hydrolysed. They are resistant to polymyxin B and colistin.

Human isolates of *Providencia* species have been recovered from urine, throat, perineum, axilla, stool, blood, and wound specimens21.

*Salmonella* species

For more information on *Salmonella* species, refer to [ID 24 - Identification of *Salmonella* species](http://www.hpa.org.uk/SMI/pdf/Identification).

*Serratia* species

The genus *Serratia* contains 15 species and 3 subspecies (but only 2 are commonly isolated from clinical material)23. They are *Serratia liquefaciens* and *Serratia marcescens*, the latter often producing a pigment called prodigiosin, which ranges in colour from dark red to pale pink, depending on the age of the colonies when grown at 20°C. Pigment production is highly variable among species and is dependent on many factors such as species type and incubation time. Non-pigmented colonies resemble other members of *Enterobacteriaceae*. The optimal growth temperature is 37°C but they can also grow in temperatures that range from 5– 40°C. They are facultative anaerobes. Most of the species are motile and have peritrichous flagella. Cells are rod shaped and 0.5-0.8 µm x 1.0-5.0 µm in diameter. Members of the genus characteristically produce three enzymes lipase, DNase and gelatinase. They are also resistant to polymyxin B and colistin and this resistance may be heterogeneous, leading to a target-zone appearance.

They are positive for glucose and sucrose (with gas production) fermentation and nitrate test; and negative for indole, urease and oxidase.

Rare reports have described disease resulting from infection with *Serratia plymuthica, Serratia liquefaciens, Serratia rubidaea, Serratia odorifera,* and *Serratia fonticola*.

*Serratia* species are found in faeces, wound exudates, respiratory specimen, blood, eye culture, and urine19.

*Serratia marcescens* is the type species.

*Shigella* species

For more information on the identification of *Shigella* species, refer to [ID 20 - Identification of *Shigella* species](http://www.hpa.org.uk/SMI/pdf/Identification).

*Yersinia* species

For more information on the identification of *Yersinia* species, refer to [ID 21 - Identification of *Yersinia* Species from Faeces](http://www.hpa.org.uk/SMI/pdf/Identification).

**Other genera of the family *Enterobacteriaceae*24-27.**

Other genera of the family reported to have caused infection are listed in section 2.

Principles of Identification

Colonial morphology, Gram’s stain, oxidase and the use of several biochemical tests identify isolates from clinical material. Enteric pathogens such as *Salmonella* species should be identified biochemically and typed serologically. *Hafnia*, *Morganella* and *Proteus* species can resemble non-motile *Salmonella* biochemically, and can agglutinate in polyvalent Salmonella antisera. Because of the diversity of biochemical activities, all the reactions of every species are not described in this SMI. Therefore only a few screening tests are included together with results for the more common genera and species.

Careful consideration should be given to isolates that give an unusual identification. All evidence including growth characteristics, cultural morphology and serology should be considered before accepting commercial identification system results.

If further identification or confirmation is required, isolates should be sent to the Reference Laboratory

Technical Information/Limitations

Indole Test

Organisms to be tested by the spot indole method must be taken from a tryptophan - containing medium (eg blood agar) and never on MacConkey agar as they have pH indicators and pigmentation of lactose-positive colonies which will make interpretation of colour reaction difficult28.

Commercial Identification System

With new species being recently established, no commercial system currently includes all species in its database and so when tested with these systems, the final identification with an unacceptable profile or no identification at all is usually generated eg *Escherichia albertii* and *Hafnia alvei*11,29.

Serotyping

Rough strains:

Serotyping should not be attempted on *Shigella* strains which autoagglutinate saline. Rough isolates seldom revert to smooth forms. However, if autoagglutination in saline is observed, an attempt to recover smooth colonies may be made by performing 2- 4 serial sub-cultures on enriched media, such as blood agar30.

Capsular antigens:

Occasionally, the presence of capsular antigens may prevent some isolates of *Shigella* species from reacting with polyvalent antisera. The presence of capsular antigens should be considered when isolates which are biochemically typical of *Shigella* spp., fail to agglutinate (or agglutinate poorly) with *Shigella* polyvalent antisera.

*Shigella sonnei* polyvalent antisera can produce cross-reactions with some cultures of *Shigella boydii* type 6 due to the presence of conserved antigens. In these situations, biochemical profile and the use of *Shigella boydii* type 6 monovalent antiserum will be necessary to confirm the identification30.

Some *Plesiomonas shigelloides* strains share antigens with *Shigella sonnei*, and cross-reactions with *Shigella* antisera occur18.

This also applies to *Klebsiella* species where cross-reactions occur among the 77 capsule types and then, individual sera have to be absorbed with the cross-reacting K-antigens. The serotyping procedure for *Klebsiella* is cumbersome because of the time needed to perform the test and is susceptible to subjective interpretations because of weak reactions that are not always easy to interpret. Since the anti-capsule antisera are not commercially available, this technique is practiced mostly in specialized laboratories13.

Misinterpreted results

When conflicting results are observed, for example; biochemical profile disagrees with serological profile, tests should be repeated from the original plate.

Quality control

Each new lot or shipment of antisera/commercial identification systems should be tested and validated for positive and negative reactivity using known control strains; ensuring it is fit for purpose. Laboratories must follow manufacturer’s instructions when using these products.

1 Safety Considerations24-27,31-43

All *Salmonella* Typhi*, Salmonella* ParatyphiA, B and C, *Shigella dysenteriae* type 1, *E. coli* O157 and *Yersinia pestis* are Hazard Group 3 organisms and suspected isolates must be handled in a containment level 3 room.

*Salmonella* species

Most *Salmonella* species are in hazard group 2 with important exceptions including *S*. Typhi and *S.* Paratyphi A, B and C. Work involving these organisms must be performed under containment level 3 conditions.

*S.* Typhi, *S*. Paratyphi A, B and C cause severe and sometimes fatal disease. 258 cases and 20 deaths due to laboratory acquired infections have been reported44,45. *S*. Typhi vaccination is available and guidance is given in the Department of Health immunisation policy46.

*Shigella* species

*Shigella* species are highly infective, particularly *S. dysenteriae*37,38. As few as 10 viable organisms are required for an infective dose and cause infection47.

*Shigella* species have been recently identified to be the most frequently identified agent of laboratory-acquired infections because of their high virulence and low infectious dose. A large number of laboratory acquired infections have been reported. Infection may be acquired through ingestion or accidental parenteral inoculation48,49.

*Escherichia* species

*Escherichia* speciesare highly infective particularly *E. coli* 0157, and as few as 10 viable organisms are required for an infective dose.

It has been identified as one of the enteric pathogens reported to cause laboratory acquired infection, but they are as less common. There are 4 reported cases of laboratory infections with *E. coli* since 198148-51.

*Yersinia* pestis

*Yersinia pestis* is highly infective and the infectious dose is of 108 bacteria or more orally(Fleming D & Hunt D, 2006).

There have been 10 reported laboratory-acquired infections with 4 deaths52. Vaccination is recommended for laboratory personnel who are routinely exposed to live *Y. pestis*.

Klebsiella species

*Klebsiella* species are Hazard group 2 organisms and have also been identified as important common pathogens for nosocomial pneumonia, septicaemia, urinary tract infection, wound infections, intensive care unit (ICU) infections, and neonatal septicaemias14. *K. pneumoniae* is most pathogenic to humans among all *Klebsiella* spp, followed by *K. oxytoca. K. ozaenae* and *K. rhinoscleromatis* cause specific diseases in humans13.

According to Janda *et al*, 108 *Klebsiella* organisms per gram of faeces are required to produce damage14. Only one case of laboratory-acquired infection with *K. pneumoniae* up to 1976 has been reported53.

*Serratia* species

*Serratia* species are Hazard Group 2 organisms and their infectious dose is unknown. However, 5 laboratory acquired infection with *S. marcescens* have been reported54.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

Appropriate personal protective equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 Target Organisms

*Enterobacteriaceae* reported to have caused human infections 11,13,14,16,21,24-26,29,50,52,55-60

|  |  |
| --- | --- |
| **Genus** | **Species** |
| *Cedecea* | *davisae, lapagei, neteri,* species 3, species 5 |
| ***Citrobacter*** | *amalonaticus, braakii, farmeri, freundii, gillenii, koseri, murliniae, sedlakii, werkmanii, youngae* |
| *Cronobacter* | *sakazakii (Enterobacter* *sakazakii) , malonaticus, turicensis, universalis* ( previously called *Cronobacter* genomospecies 1) |
| *Edwardsiella* | *hoshinae, ictaluri, tarda* |
| ***Enterobacter*** | *aerogenes, amnigenus, asburiae,* *cancerogenus*, *cloacae, cowanii, gergoviae, hormaechei, kobei, ludwigii,* |
| ***Escherichia*** | *albertii, coli, fergusonii, hermanii, vulneris,* |
| *Ewingella* | *Americana* |
| ***Hafnia*** | *alvei, paralvei* (previously known as *Hafnia alvei* biogroup 2) |
| ***Klebsiella*** | *granulomatis, oxytoca, pneumoniae* subspecies *ozaenae, pneumoniae, and rhinoscleromatis, variicola* |
| *Kluyvera* | *ascorbata, cryocrescens, georgiana* |
| *Leclercia* | *adecarboxylata* |
| ***Morganella*** | *morganii subspecies morganii and sibonii* |
| *Pantoea* | *agglomerans, dispersa, septica,* |
| *Photorhabdus* | *luminescens, asymbiotica* subspecies *australis, asymbiotica* |
| ***Plesiomonas*** | *Shigelloides* |
| ***Proteus*** | *mirabilis, penneri, vulgaris* |
| ***Providencia*** | *alcalifaciens, rettgeri, stuartii* |
| *Rahnella* | *Aquatilis* |
| ***Salmonella*** | *bongori, enterica (>2500 serotypes)* - subspecies *arizonae, diarizonae, enterica, houtenae, indica* and *salamae,* main serotypes (serovars) *-* Enteritidis*,* Paratyphi*,* Typhi, Typhimurium. |
| ***Serratia*** | *fonticola, grimesii, liquefaciens, marcescens, odorifera, plymuthica, proteamaculans, quinovorans, rubidaea* |
| ***Shigella*** | *boydii, dysenteriae, flexneri, sonnei* |
| *Tatumella* | *Ptyseos* |
| ***Yersinia*** | *aldovae, bercovieri, enterocolitica, intermedia, frederiksenii, kristensenii, mollaretti, pestis, pseudotuberculosis, rohdei* |
| *Yokenella* | *Regensburgei* |

Other genera of the family *Enterobacteriaceae* that may have been associated with human infections61-63

*Citrobacter gillenii (*Formerly *Citrobacter Genomospecies* 10*), Citrobacter murliniae (*Formerly *Citrobacter Genomospecies* 11*), Leminorella grimontii, Leminorella richardii, Moellerella wisconsensis*,

Other genera and species of the *Enterobacteriaceae* may rarely be associated with human disease.

3 Identification

3.1 Microscopic Appearance

Gram stain ([TP 39 - Staining Procedures](http://www.hpa.org.uk/SMI/pdf/Testprocedures))

Gram negative rods, some may show bipolar staining (eg *Yersinia* species).

3.2 Primary Isolation Media

Blood agar (BA): 16-24hr incubation in 5-10% CO2 at 35-37°C.

MacConkey (MAC) agar: 16–24hr incubation in air at 35-37°C.

Cystine-lactose-electrolyte deficient (CLED) agar with bromothymol blue (CLED B) or Andrade’s indicator (CLED A): 16–24hr incubation in air at 35-37°C.

Selective enteric media, incubation in air at 35-37°C for 16–24hr:

Desoxycholate citrate agar (DCA).

Xylose-lysine-desoxycholate agar (XLD).

Brilliant Green Agar (BGA)

Cefixime-tellurite-sorbitol-MacConkey (CT-SMAC) agar.

Thiosulphate-citrate-bile salt (TCBS) agar.

Cefsulodin-Irgasan (triclosan)-novobiocin (CIN) agar incubated in air at 32°C for 24–48hr.

Chromogenic media incubated in air at 35-37°C for 16-24hr.

3.3 Colonial Appearance

**BA**– Colonies are 2-3mm diameter, low, convex, grey, smooth or mucoid, may be haemolytic or swarming.

**MAC**– Colonies may appear pink (lactose fermenting) or colourless (lactose non-fermenting), size and shape vary with individual species.

**CLED B**– Colonies may appear yellow (lactose fermenting) or blue (lactose non-fermenting), size and shape vary with individual species.

**CLED A**- Colonies may appear pink (lactose fermenting) or green, translucent (lactose non-fermenting), size and shape vary with individual species.

**DCA**– Colonies may appear pink (lactose fermenting) or colourless (lactose non-fermenting) and may have black centre (H2S producers).

**XLD**– Colonies may appear yellow (xylose, lactose or sucrose fermenting) or pink (non-fermenting) and may have black centre (H2S producers).

**BGA-** Colonies appear as red-pink, 1-3mm in diameter, surrounded by brilliant red zones in the agar.

**CT-SMAC**– Colonies may appear pink (sorbitol fermenting) or colourless (sorbitol non-fermenting).

**TCBS**– Colonies may appear yellow (sucrose fermenting) or blue-green (sucrose non-fermenting).

**CIN**– Colonies may have deep-red centres (mannitol fermenting) surrounded by a translucent border giving the appearance of a “bull’s eye”.

**Note:** Colonies of *Yersinia* species may be smaller than those of other *Enterobacteriaceae*.

3.4 Test Procedures

Oxidase Test ([TP 26 - Oxidase Test](http://www.hpa.org.uk/SMI/pdf/testprocedures))

All *Enterobacteriaceae* are oxidase negative.

Indole Test [(TP19 – Indole Test)](http://www.hpa.org.uk/smi/pdf/testprocedures) - Optional

The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan. It is an aid in differentiation of the *Enterobacteriaceae* and other genera.

*Klebsiella, Enterobacter, Hafnia* and *Serrat*ia species give variable indole reactions but are usually negative.

*Escherichia* species are positive for indole except *E. vulneris.*

Carbohydrates Fermentation Test

Lactose fermentation exhibits variable results depending on the genus and species.

Commercial identification kit

Laboratories must follow manufacturer’s instructions and rapid tests and kits must be validated and be shown to be fit for purpose prior to use.

Serotyping

Serotyping is subtyping method based on the immuno-reactivity of various antigens. *Shigella* species are by definition non-motile, as such, only the somatic (O) antigens are utilized for the determination of serotype. Flagellar (H) antigens are not expressed.

The majority of serotypes of *Salmonella* possess two phases of H (flagellar) antigens and serotyping of these species can also be done. Refer to [TP 3 - Agglutination Test for *Salmonella* species](http://www.hpa.org.uk/smi/pdf/testprocedures) and [TP 32 - Changing the Phase of *Salmonella*](http://www.hpa.org.uk/smi/pdf/testprocedures) for the detailed procedures.

3.5 Further Identification

Rapid Molecular Methods

Molecular methods have had an enormous impact on the taxonomy of *Enterobacteriaceae*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Enterobacteriaceae* and related organisms; and has resulted in the recognition of numerous new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of rapid identification and sensitivity methods have been developed for isolates from clinical samples; these include molecular techniques such as Real-time Polymerase Chain reaction (PCR), Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable-Number Tandem-Repeat Analysis (MVLA), SNP assays, Whole Genome Sequencing (WGS) and Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF) Mass Spectrometry. All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-TOF) Mass Spectrometry

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS), which can be used to analyse the protein composition of a bacterial cell, has emerged as a new technology for species identification. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use64.

This has been utilized to aid in both the detection and species-level identification of *Salmonella* species and *Enterobacter cloacae* complex. As a tool for subspecies and serovar typing, MALDI-TOF MS shows significant promise but will require additional studies and modifications to existing protocols before the method can be used as a stand-alone mechanism65.

This technique has equally been used for the identification of *Pantoea* species but the database would benefit from additional entries being added to further populate it with entries of both environmental and clinical interest66.

It has also been used successfully for the identification and characterization of the *Proteus mirabilis*, *Plesiomonas shigelloides* and *Enterobacter cloacae* isolates except that an improved MALDI-TOF MS database needs to be compiled in order to be able to determine species-level identifications, eg the *Enterobacter cloacae* complex65.

MALDI-TOF MS has the ability to accurately discriminate between the two clinically relevant and highly genetically similar organisms with identical 16S rRNA gene sequences, *Y. pestis* and *Y. pseudotuberculosis*67. The methods of inactivation used for these pathogenic organisms does not have any influence on the on the MALDI-TOF MS spectra generated. This has also been used to identify and subtype *Yersinia enterocolitica* isolates68.

MALDI-TOF MS is able to derive genus- and species-level identifications for *Cronobacter* isolates or identify them as non-*Cronobacter* isolates in the case of non-target strains and to identify different biovars within the *C. sakazakii* species but according to study by Cetinkaya *et al*., molecular techniques such as 16S rRNA and *fusA* gene sequencing and multilocus sequence typing (MLST) are more reliable mechanisms of *Cronobacter* identification69-71.

One of the limitations is the current inability of MALDI-TOF MS to reliably distinguish pathogenic from non-pathogenic *E. coli* isolates, in addition, numerous reports have also described the difficulty encountered when trying to discriminate *E. coli* from *Shigella* species. Differentiation of pathogenic *E. coli* strains from *Shigella* species is challenging because of the close genetic relatedness of the organisms65.

Real-time Polymerase Chain reaction (RT-PCR)

PCR is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations. Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

This has been used successfully in the identification of *Enterobacteriaceae - Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Citrobacter species, Enterobacter cloacae* and *Salmonella subspecies I, Salmonella enterica* serovars *Typhimurium, Typhi* and *Enteritidis* as well as *Salmonella enterica* subspecies *arizonae* and *diarizonae* (rapidly and accurately without the need for serological testing)72-75. However, multiplex PCR is cumbersome and sometimes lacks reproducibility between laboratories because of the specific conditions needed for simultaneous amplification of several regions76.

PCR has also been used to detection of *Y. pestis* among pathogenic *Yersinia* species and other enterobacteriaceae having antigens common to *Y. pestis*. It is very useful in cases of emergency and for the surveillance of epidemics77.

PCR has also been used to detect virulence genes of *Yersinia enterocolitica* and *Y. pseudotuberculosis* in human clinical isolates78.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories79,80.

This has been used successfully to identify and discriminate between species of the family *Enterobacteriaceae* – for example, it has been used in tracking the source of *Salmonella* infections for different serotypes and is considered the gold standard for *Salmonella* molecular typing and also for typing of *Klebsiella* species13,81,82.

Multilocus Sequence Typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the protein product. One of the advantages of MLST over other molecular typing methods is that sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the Internet83.

MLST has been extensively used as the one of the main typing methods for analysing the genetic relationships within the *Enterobacteriaceae* population especially the genus *Salmonella*81,82. MLST was found to provide better discrimination of *Salmonella* serotype Enteritidis strains than PFGE and accurately differentiate outbreak strains and clones of the *Salmonella* serovars most commonly associated with human disease. It has also been useful for typing non-typhoidal *Salmonella* strains84.

This has also been used to identify Shiga-toxin producing *E. coli* and *Shigella* species as well as *Klebsiella* species13,85.

The drawbacks of MLST are the substantial cost and laboratory work required to amplify, determine, and proofread the nucleotide sequence of the target DNA fragments, making the method hardly suitable for routine laboratory testing.

Multiple-Locus Variable-Number Tandem-Repeat Analysis (MVLA) also known as VNTR

Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms. It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms. The molecular typing profiles are used to study transmission routes, to assess sources of infection and also to assess the impact of human intervention such as vaccination and use of antibiotics on the composition of bacterial populations.

This has been used successfully in the subtyping of *Salmonella* *enterica* subsp *enterica* serovar Typhimurium, Enteritidis, Typhi, Infantis, Newport, Paratyphi A, Saintpaul, and Gallinarum isolates81,84,86,87. The method has proven very useful for detecting and investigating outbreaks, since it has the capacity to differentiate closely related strains. It is technically simple and inexpensive to perform. However, it has no usefulness for serovar assignment or for global phylogenetic studies because the scope of each MLVA is commonly restricted to a unique serovar.

This has also been used successfully to identify *Shigella* and *Escherichia* strains, suggesting that it could significantly contribute to epidemiological trace-back analysis of *Shigella* infections and pathogenic *Escherichia* outbreaks. It has also been used to genotype *Yersinia pestis*88.

Whole Genome Sequencing (WGS)

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology, IIIumina sequencing, Ion Torrent sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

This has been used successfully to explore the genome of *Shigella* species and *E. coli* O157:H7 to identify candidate genes responsible for pathogenesis, and to develop better methods of strain detection and to advance the understanding of the evolution of *E. coli.* With this technique, lateral gene transfer of *E. coli* that was discovered was very extensive47,89.

WGS has also been used to provide important insights into the pathology of *Yersinia enterocolitica* and, more broadly, into the evolution of the genus and other human enteropathogens90.

This technique has equally been used to characterise *Salmonella enterica* serovar Typhi and to discover its recently acquired genes, such as those encoding the Vi antigen, by horizontal transfer events and it has provided new insights into how this pathogen has evolved to cause invasive disease in humans82.

*rpoB* Single Nucleotide Polymorphism (*rpoB* SNP) assay

*rpoB* gene is a single-copy chromosomal gene encoding the RNA polymerase β-subunit. This gene has been previously used in phylogenetic analysis for bacteria species and genus delineation, since it is highly conserved across organisms91. However, the 16SrRNA gene has been used widely and its usefulness has been greatly enhanced through the establishment of public domain databases but its sensitivity has been questioned particularly among *Enterobacteriaceae* and so when the *rpoB* gene was used as an alternative for detection based on a Single Nucleotide Polymorphism, it was found to be more compatible with the currently accepted classification of *Enterobacteriaceae* and a powerful identification tool which may be useful for universal bacterial identification. This has been used to demonstrate that the genus *Klebsiella* is polyphyletic and to detect *Salmonella enterica* serotype Typhimurium76.

The distinct advantage of SNP and other nucleotide sequence-based methods over profile-generating methods is that genetic relationships can be established on the basis of discrete data that are directly suitable for biocomputing and statistical analysis81.

3.6 Storage and Referral

Save the pure isolate on a nutrient agar slope for referral to the Reference Laboratory.

4 Identification of *Enterobacteriaceae* Flowchart



The flowchart is for guidance only.

5 Reporting

5.1 Presumptive Identification

If appropriate growth characteristics, colonial appearance, Grams stain on pure culture, oxidase and serological results are demonstrated.

5.2 Confirmation of Identification

Further biochemical tests and/or molecular methods and/or reference laboratory report.

5.3 Medical Microbiologist

Inform the medical microbiologist of presumptive and confirmed *Y. pestis*, *S.* Typhi,   
*S.* Paratyphi, *Shigella* species, *E. coli* O157 and *Salmonella* species (according to local procedures).

The medical microbiologist should also be informed if the request card bears information relating to infection with *Y. pestis* eg

* Ulceroglandular/pneumonic syndrome
* Septicaemia
* Travelling, hunting, farming, or veterinary work overseas

Information relating to cases of:

* Enterocolitis
* Dysentery
* Septicaemia
* Haemolytic-uraemic syndrome
* Neurological dysfunction or confusional states
* (non-blanching) rash

Presumptive or confirmed agents of enteric fever, dysentery, and enterocolitis should also be relayed to the medical microbiologist, especially if the patient has a history of:

* recent foreign travel
* farming (or visits to farms)
* veterinary or laboratory work
* alcoholism, substance abuse, immunodeficiency or other serious underlying disorder such as cancer

Presumptive and confirmed isolates of *Enterobacteriaceae* from cases of food poisoning and from investigations of outbreak situations should additionally be reported to the medical microbiologist.

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding**.**

All clinically significant isolates should be notified by the diagnostic laboratories to ensure urgent initiation of proper procedures.

<http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook/fs/en>

5.5 Public Health England92-97

Refer to current guidelines on CDSC and COSURV reporting.

From 1 October 2010 provisions relating to diagnostic laboratories come into force. The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAIs and CJD under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

Other arrangements exist in Scotland94,95, Wales96 and Northern Ireland97.

Notify all isolates of the following:

*E. coli* (presumptive [locally-confirmed] VTEC O157 and other possible VTEC strains)

*Salmonella* species

*Shigella* species

*Yersinia pestis*

**Urgent oral notification to the Public Health England Centre within 24hr of identification is likely to be necessary to protect human health when presumptive identification is made of the following**:

*S.* Typhi or *S*. Paratyphi

*Salmonella* species, if a suspected outbreak or a case in a food handler or closed community such as a care home

*Shigella* species other than *S. sonnei*

*S. sonnei*, if a suspected outbreak or a case in a food handler or closed community such as a care home

*E. coli* O157 when presumptive (locally confirmed) at the diagnostic laboratory

Other verocytotoxigenic *E. coli* O157

*Yersinia pestis*

**Confirmatory and typing results should be forwarded to the Public Health England Centre as soon as they are available to expedite appropriate health protection interventions.**

5.6 Infection Control Team

Inform the infection control team of presumptive and confirmed isolates of *E. coli* O157, *Yersinia*, *Salmonella* and *Shigella* species.

6 Referrals

6.1 Reference Laboratory

Contact appropriate devolved nation reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales <http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>

Gastrointestinal Infections Reference Unit

Microbiology Services

Public Health England

61 Colindale Avenue

London

NW9 5EQ

Contact PHE’s main switchboard: Tel. +44 (0) 20 8200 4400

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

References

1. Hong Nhung P, Ohkusu K, Mishima N, Noda M, Monir Shah M, Sun X, et al. Phylogeny and species identification of the family Enterobacteriaceae based on dnaJ sequences. Diagnostic Microbiology and Infectious Disease 2007;58:153-61.

2. Iversen C, Lehner A, Mullane N, Bidlas E, Cleenwerck I, Marugg J, et al. The taxonomy of Enterobacter sakazakii: proposal of a new genus Cronobacter gen. nov. and descriptions of Cronobacter sakazakii comb. nov. Cronobacter sakazakii subsp. sakazakii, comb. nov., Cronobacter sakazakii subsp. malonaticus subsp. nov., Cronobacter turicensis sp. nov., Cronobacter muytjensii sp. nov., Cronobacter dublinensis sp. nov. and Cronobacter genomospecies 1. BMC Evol Biol 2007;7:64.

3. Ewing WH, Farmer JJ, III, Brenner DJ. Proposal of *Enterobacteriaceae* fam.nov., nom. rev. to replace *Enterobacteriaceae* Rahn 1937, nom. fam. cons. (Opin. 15, Jud. Comm. 1958), which lost standing in nomenclature on 1 January 1980. International Journal of Systematic Bacteriology 1980;30:674-5.

4. Holt JG, Krieg N R, Sneath P H A, Staley J T, Williams S T, editors. Bergey's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins; 1994. p. 175-222

5. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Citrobacter*.

6. MacFaddin JF. Gram - Negative *Enterobacteriaceae* and other Intestinal Bacteria. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 732-802.

7. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Cenus *Enterobacter*.

8. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Escherichia*.

9. Euzeby,JP. List of Prokaryotic names with Standing in Nomenclature - Genus *Hafnia*.

10. Winstanley TG, Limb DI, Wheat PF, Nicol CD. Multipoint identification of Enterobacteriaceae: report of the British Society for Microbial Technology collaborative study. J Clin Pathol 1993;46:637-41.

11. Janda JM, Abbott SL. The genus Hafnia: from soup to nuts. Clin Microbiol Rev 2006;19:12-8.

12. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Klebsiella*.

13. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11:589-603.

14. Janda JM, Abbott SL. The Genera *Klebsiella* and *Roaultella*. The Enterobacteria. 2 ed. Washington, USA: ASM Press; 2006. p. 115-29.

15. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Morganella*.

16. Jensen KT, Frederiksen W, Hickman-Brenner FW, Steigerwalt AG, Riddle CF, Brenner DJ. Recognition of Morganella subspecies, with proposal of Morganella morganii subsp. morganii subsp. nov. and Morganella morganii subsp. sibonii subsp. nov. Int J Syst Bacteriol 1992;42:613-20.

17. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Plesiomonas*.

18. Niedziela T, Lukasiewicz J, Jachymek W, Dzieciatkowska M, Lugowski C, Kenne L. Core oligosaccharides of Plesiomonas shigelloides O54:H2 (strain CNCTC 113/92): structural and serological analysis of the lipopolysaccharide core region, the O-antigen biological repeating unit, and the linkage between them. J Biol Chem 2002;277:11653-63.

19. Abbott SL. *Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas, a*nd other *Enterobacteriaceae*. In: Murray, editor. Manual of Clinical Microbiology. 9 ed. Washington: ASM; 2007. p. 698-715.

20. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Proteus*.

21. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev 2000;13:534-46.

22. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Providencia*.

23. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Serratia*.

24. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.

25. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.

26. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

27. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.

28. MacFaddin JF. Indole Test. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 221-32.

29. Abbott SL, O'Connor J, obin T, immer BL, anda JM. Biochemical properties of a newly described *Escherichia* species, *Escherichia albertii*. Journal of Clinical Microbiology 2003;41:4852-4.

30. Ploeg van der,CA, Vinas,MR, erragno,R et al . Laboratory protocol: "Serotyping of *Shigella* spp.". p. 1-24.

31. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

32. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.

33. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.

34. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.

35. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.

36. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).

37. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32

38. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.

39. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.

40. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.

41. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.

42. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.

43. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14

44. Blaser MJ, Lofgren JP. Fatal salmonellosis originating in a clinical microbiology laboratory. J Clin Microbiol 1981;13:855-8.

45. Harding AL, Byers KB. Epidemiology of laboratory-associated infections. In: Fleming D, Hunt D, editors. Biology safety: principles and practices. 4 ed. Washington DC, USA: ASM press; 2006. p. 53-77.

46. Salisbury D, Ramsay M, Noakes K, editors. Immunisation against infectious disease 2006 - The Green Book. Updated 17 July 2013. 3rd ed. Great Britain: The Stationery Office; 2013. p. 1-514

47. Peng J, Yang J, Jin Q. The molecular evolutionary history of Shigella spp. and enteroinvasive Escherichia coli. Infection, Genetics and Evolution 2009;9:147-52.

48. Singh K. Laboratory-acquired infections. Clin Infect Dis 2009;49:142-7.

49. Baron EJ, Miller JM. Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. 3 2008;60:241-6.

50. Spina N, Zansky S, Dumas N, Kondracki S. Four laboratory-associated cases of infection with Escherichia coli O157:H7. J Clin Microbiol 2005;43:2938-9.

51. Burnens AP, Zbinden R, Kaempf L, Heinzer I, Nicolet J. A case of laboratory acquired infection with Escherichia coli O157:H7. Zentralbl Bakteriol 1993;279:512-7.

52. Ritger K, Black S, Weaver K, Jones J, Gerber S, et al. Fatal laboratory- acquired Infection with an Attenuated Yersinia pestis Strain - Chicago, IIIinois, 2009. MMWR 2011;60:201-5.

53. Pike RM. Laboratory-associated infections: summary and analysis of 3921 cases. Health Lab Sci 1976;13:105-14.

54. Collins CH, Kennedy.D.A. Laboratory acquired infections. In: Woburn MA, editor. Laboratory acquired infection: History, incidence, causes and prevention. 4 ed. 1999. p. 1-37.

55. Foti M, Daidone A, Aleo A, Pizzimenti A, Giacopello C, Mammina C. Salmonella bongori 48:z35:- in migratory birds, Italy. Emerg Infect Dis 2009;15:502-3.

56. Iversen C, Mullane N, McCardell B, Tall BD, Lehner A, Fanning S, et al. Cronobacter gen. nov., a new genus to accommodate the biogroups of Enterobacter sakazakii, and proposal of Cronobacter sakazakii gen. nov., comb. nov., Cronobacter malonaticus sp. nov., Cronobacter turicensis sp. nov., Cronobacter muytjensii sp. nov., Cronobacter dublinensis sp. nov., Cronobacter genomospecies 1, and of three subspecies, Cronobacter dublinensis subsp. dublinensis subsp. nov., Cronobacter dublinensis subsp. lausannensis subsp. nov. and Cronobacter dublinensis subsp. lactaridi subsp. nov. Int J Syst Evol Microbiol 2008;58:1442-7.

57. Stock I, Sherwood KJ, Wiedemann B. Antimicrobial susceptibility patterns, [beta]-lactamases, and biochemical identification of Yokenella regensburgei strains. Diagnostic Microbiology and Infectious Disease 2004;48:5-15.

58. Chang CL, Jeong J, Shin JH, Lee EY, Son HC. Rahnella aquatilis sepsis in an immunocompetent adult. J Clin Microbiol 1999;37:4161-2.

59. Farmer JJ, III, Davis BR, Hickman-Brenner FW, McWhorter A, Huntley-Carter GP, Asbury MA, et al. Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. J Clin Microbiol 1985;21:46-76.

60. Akhurst RJ, Boemare NE, Janssen PH, Peel MM, Alfredson DA, Beard CE. Taxonomy of Australian clinical isolates of the genus Photorhabdus and proposal of Photorhabdus asymbiotica subsp. asymbiotica subsp. nov. and P. asymbiotica subsp. australis subsp. nov. Int J Syst Evol Microbiol 2004;54:1301-10.

61. Hickman-Brenner FW, Huntley-Carter GP, Saitoh Y, Steigerwalt AG, Farmer JJ, III, Brenner DJ. Moellerella wisconsensis, a new genus and species of Enterobacteriaceae found in human stool specimens. J Clin Microbiol 1984;19:460-3.

62. Hickman-Brenner FW, Vohra MP, Huntley-Carter GP, Fanning GR, Lowery VA, III, Brenner DJ, et al. Leminorella, a new genus of Enterobacteriaceae: identification of Leminorella grimontii sp. nov. and Leminorella richardii sp. nov. found in clinical specimens. J Clin Microbiol 1985;21:234-9.

63. Brenner DJ, O'Hara CM, Grimont PA, Janda JM, Falsen E, Aldova E, et al. Biochemical identification of Citrobacter species defined by DNA hybridization and description of Citrobacter gillenii sp. nov. (formerly Citrobacter genomospecies 10) and Citrobacter murliniae sp. nov. (formerly Citrobacter genomospecies 11). J Clin Microbiol 1999;37:2619-24.

64. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, et al. Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 2008;74:5402-7.

65. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 2013;26:547-603.

66. Rezzonico F, Vogel G, Duffy B, Tonolla M. Application of whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification and clustering analysis of pantoea species. Appl Environ Microbiol 2010;76:4497-509.

67. Wittwer M, Heim J, Schar M, Dewarrat G, Schurch N. Tapping the potential of intact cell mass spectrometry with a combined data analytical approach applied to Yersinia spp.: detection, differentiation and identification of Y. pestis. Syst Appl Microbiol 2011;34:12-9.

68. Stephan R, Cernela N, Ziegler D, Pfluger V, Tonolla M, Ravasi D, et al. Rapid species specific identification and subtyping of Yersinia enterocolitica by MALDI-TOF mass spectrometry. J Microbiol Methods 2011;87:150-3.

69. Stephan R, Ziegler D, Pfluger V, Vogel G, Lehner A. Rapid genus- and species-specific identification of Cronobacter spp. by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010;48:2846-51.

70. Cetinkaya E, Joseph S, Ayhan K, Forsythe SJ. Comparison of methods for the microbiological identification and profiling of Cronobacter species from ingredients used in the preparation of infant formula. Mol Cell Probes 2013;27:60-4.

71. Karamonova L, Junkova P, Mihalova D, Javurkova B, Fukal L, Rauch P, et al. The potential of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the identification of biogroups of Cronobacter sakazakii. Rapid Commun Mass Spectrom 2013;27:409-18.

72. Anbazhagan D, Kathirvalu GG, Mansor M, Yan GOS, Yusof, M.Y., et al. Multiplex polymerase chain reaction (PCR) assays for the detection of *Enterobacteriaceae* in clinical samples. African Journal of Microbiology Research 2010;4:1186-91.

73. Park SH, Kim HJ, Cho WH, Kim JH, Oh MH, Kim SH, et al. Identification of Salmonella enterica subspecies I, Salmonella enterica serovars Typhimurium, Enteritidis and Typhi using multiplex PCR. FEMS Microbiol Lett 2009;301:137-46.

74. Hopkins KL, Lawson AJ, Connell S, Peters TM, de PE. A novel real-time polymerase chain reaction for identification of Salmonella enterica subspecies enterica. Diagn Microbiol Infect Dis 2011;70:278-80.

75. Hopkins KL, Peters TM, Lawson AJ, Owen RJ. Rapid identification of Salmonella enterica subsp. arizonae and S. enterica subsp. diarizonae by real-time polymerase chain reaction. Diagn Microbiol Infect Dis 2009;64:452-4.

76. Guijarro HK, Feingold SE, Terzolo HR. A single nucleotide polymorphism on *rpoB* gene allows specific identification of - *Salmonella enterica* serotype typhimurim`. Research Journal of Microbiology 2012;7:344-52.

77. Tsukano H, Itoh K, Suzuki S, Watanabe H. Detection and identification of Yersinia pestis by polymerase chain reaction (PCR) using multiplex primers. Microbiol Immunol 1996;40:773-5.

78. Thoerner P, Bin Kingombe CI, Bogli-Stuber K, Bissig-Choisat B, Wassenaar TM, Frey J, et al. PCR detection of virulence genes in Yersinia enterocolitica and Yersinia pseudotuberculosis and investigation of virulence gene distribution. Appl Environ Microbiol 2003;69:1810-6.

79. Liu D. Identification, subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. J Med Microbiol 2006;55:645-59.

80. Brosch R, Brett M, Catimel B, Luchansky JB, Ojeniyi B, Rocourt J. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of listeria monocytogenes via pulsed-field gel electrophoresis (PFGE). Int J Food Microbiol 1996;32:343-55.

81. Wattiau P, Boland C, Bertrand S. Methodologies for Salmonella enterica subsp. enterica subtyping: gold standards and alternatives. Appl Environ Microbiol 2011;77:7877-85.

82. Baker S, Dougan G. The genome of Salmonella enterica serovar Typhi. Clin Infect Dis 2007;45 Suppl 1:S29-S33.

83. Feil EJ, Spratt BG. Recombination and the population structures of bacterial pathogens. Annu Rev Microbiol 2001;55:561-90.

84. Liu F, Barrangou R, Gerner-Smidt P, Ribot EM, Knabel SJ, Dudley EG. Novel virulence gene and clustered regularly interspaced short palindromic repeat (CRISPR) multilocus sequence typing scheme for subtyping of the major serovars of Salmonella enterica subsp. enterica. Appl Environ Microbiol 2011;77:1946-56.

85. Choi SY, Jeon YS, Lee JH, Choi B, Moon SH, von SL, et al. Multilocus sequence typing analysis of Shigella flexneri isolates collected in Asian countries. J Med Microbiol 2007;56:1460-6.

86. Octavia S, Lan R. Multiple-locus variable-number tandem-repeat analysis of Salmonella enterica serovar Typhi. J Clin Microbiol 2009;47:2369-76.

87. Lindstedt BA, Heir E, Gjernes E, Kapperud G. DNA fingerprinting of Salmonella enterica subsp. enterica serovar typhimurium with emphasis on phage type DT104 based on variable number of tandem repeat loci. J Clin Microbiol 2003;41:1469-79.

88. Gorge O, Lopez S, Hilaire V, Lisanti O, Ramisse V, Vergnaud G. Selection and validation of a multilocus variable-number tandem-repeat analysis panel for typing Shigella spp. J Clin Microbiol 2008;46:1026-36.

89. Perna NT, Plunkett G, III, Burland V, Mau B, Glasner JD, Rose DJ, et al. Genome sequence of enterohaemorrhagic Escherichia coli O157:H7. Nature 2001;409:529-33.

90. Thomson NR, Howard S, Wren BW, Holden MT, Crossman L, Challis GL, et al. The complete genome sequence and comparative genome analysis of the high pathogenicity Yersinia enterocolitica strain 8081. PLoS Genet 2006;2:e206.

91. Mollet C, Drancourt M, Raoult D. rpoB sequence analysis as a novel basis for bacterial identification. Mol Microbiol 1997;26:1005-11.

92. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37

93. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.

94. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).

95. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.

96. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.

97. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).

1. #Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology. [↑](#footnote-ref-1)