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What’s New in Group A streptococcus (GAS) and Invasive GAS Disease Research in Ontario

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Learning objectives

1. Describe epidemiology of invasive and non-invasive Group A Streptococcus (GAS) in children
2. Describe recent changes in the incidence of iGAS in Toronto and the Peel region
3. Review the incidence and epidemiology of iGAS in homeless persons
4. Determine the viability of whole genome sequencing to differentiate invasive from non-invasive GAS clinical isolates

Comparison of pharyngeal and invasive isolates of *Streptococcus pyogenes* by whole genome sequencing

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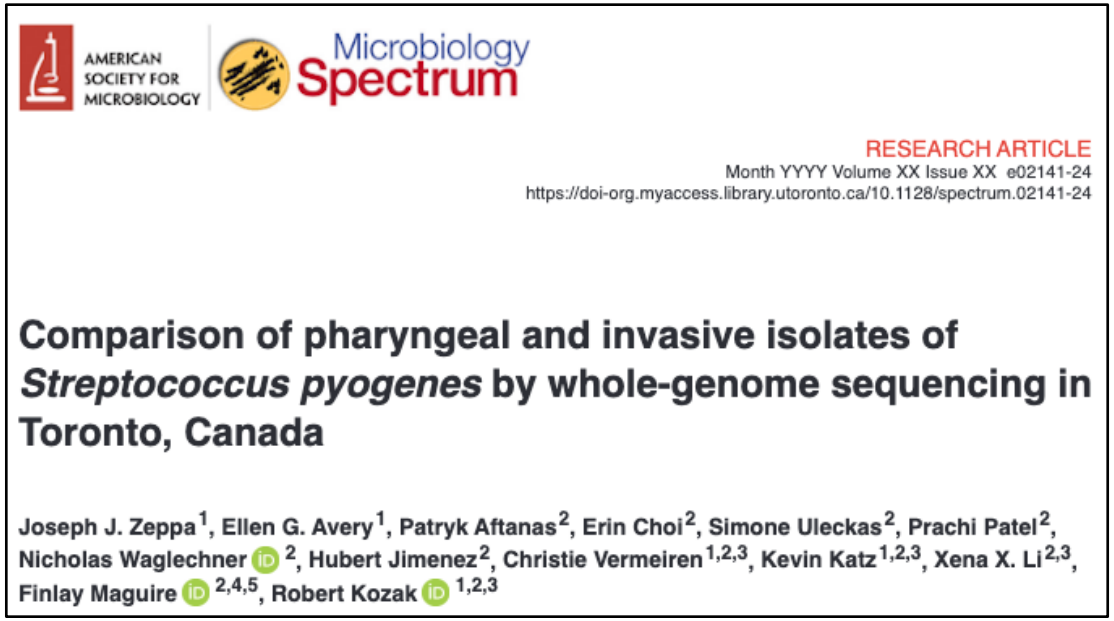
Conflicts of Interest/Disclosures

- None

Overview

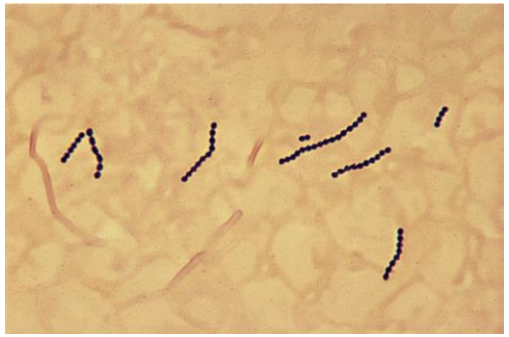
- Background on Group A Streptococcus (*S. pyogenes*)

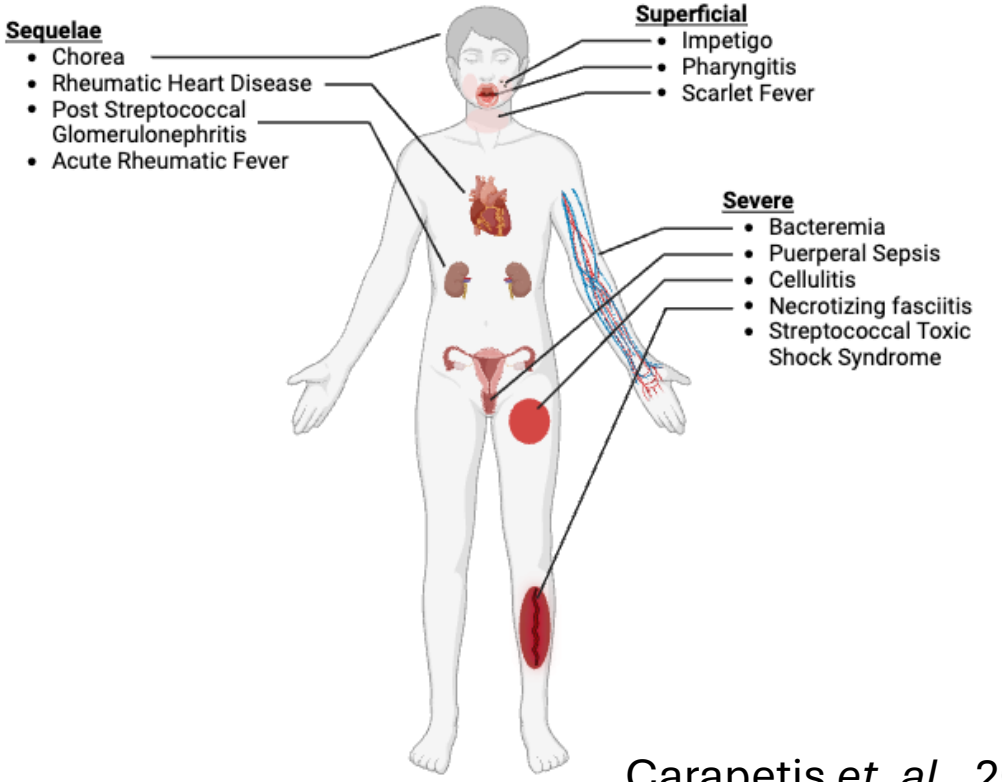
- Findings from our recently published study:



Streptococcus pyogenes

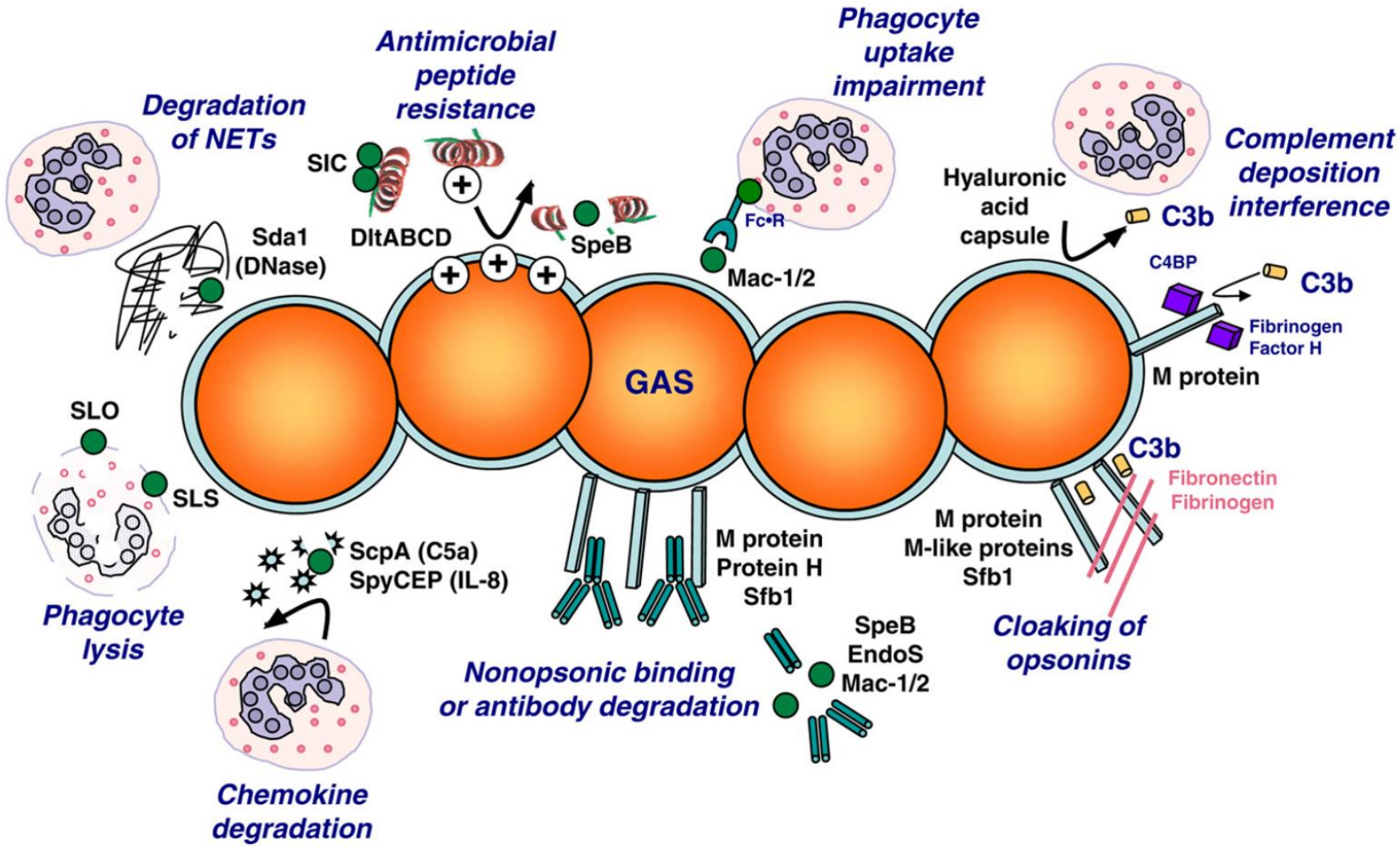
- Gram-positive, human restricted pathogen
- Capable of infecting/colonizing almost any tissue in the body
- Causing a wide variety of disease manifestations
  - Asymptomatically colonizes ~12% of school-aged children
  - 600 million cases of pharyngitis
  - 100 million skin infections
  - **500,000 deaths/year**





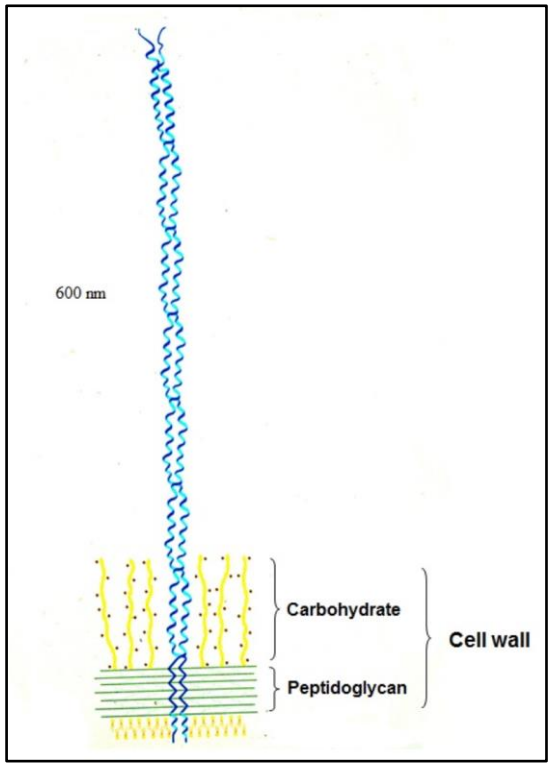
Carapetis *et. al.*, 2005
Shaikh *et. al.*, 2010

Group A Streptococcus Virulence Factors



Walker *et. al*. 2014

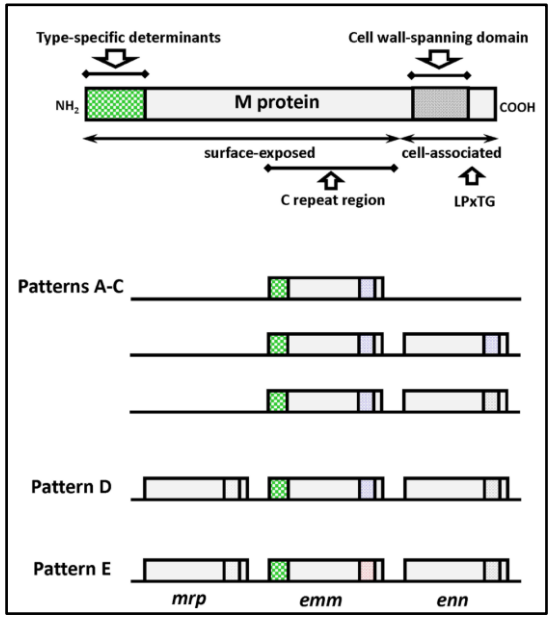
M protein and GAS typing



M protein

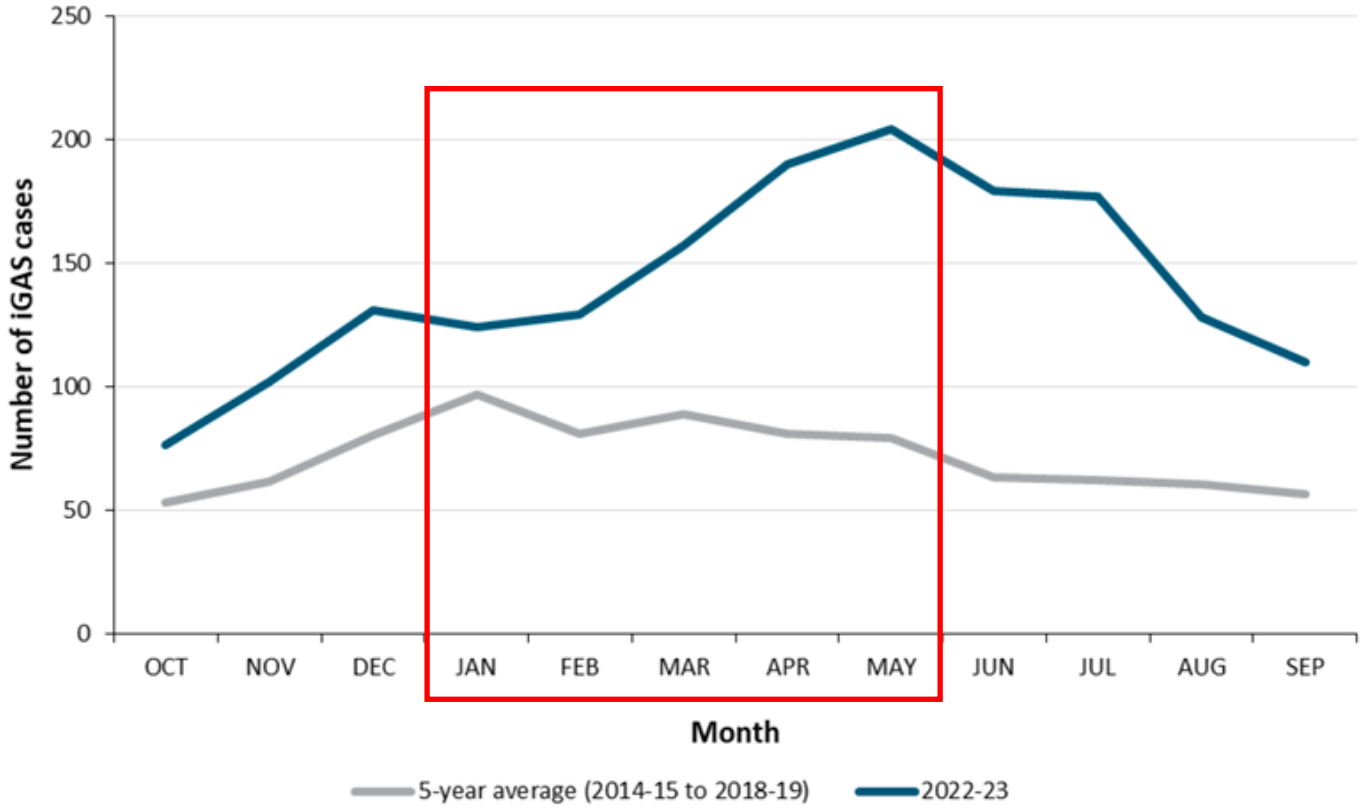
- Surface bound, antiphagocytic virulence factor
- Used in typing:

Scheme	Method	Number
M serotyping	• Immunoprecipitation using rabbit serum	>80
emm typing	• Sequencing first 30 codons (90 bp) of mature M protein • >92% similarity = same *emm* type	> 275
emm subtyping	• Sequencing first 50 codons (150bp) of mature M protein plus 10 terminal COOH codons (30bp) = 180bp • Any change to 180bp sequence = new *emm* subtype	>1900



Ontario, Canada – 2022 - 2023

Figure 2. Confirmed iGAS case counts by month: 2022-23 season (October 1, 2022 – September 30, 2023) compared to five pre-pandemic seasons (October 1, 2014 – September 30, 2019)



Data source: Ontario. Ministry of Health; 2024.[4]

Can we use whole genome sequencing to determine if there is a genomic change that can account for this trend?

Methodology

① **Sample Collection**
January - May, 2023

Non-Invasive (Throat)

N = 117

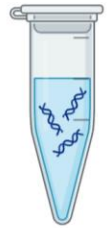
Invasive (Blood)

N = 38

② **Isolate Bacteria**

Group A Streptococcus

③ **Extract DNA**

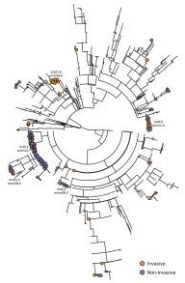


④ **Whole Genome Sequencing**

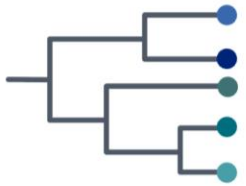
Illumina MiniSeq

⑤ **Analysis**

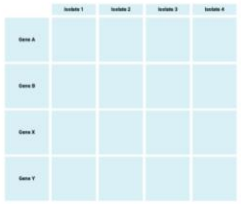
Minimum Spanning Tree



Core Genome Phylogeny

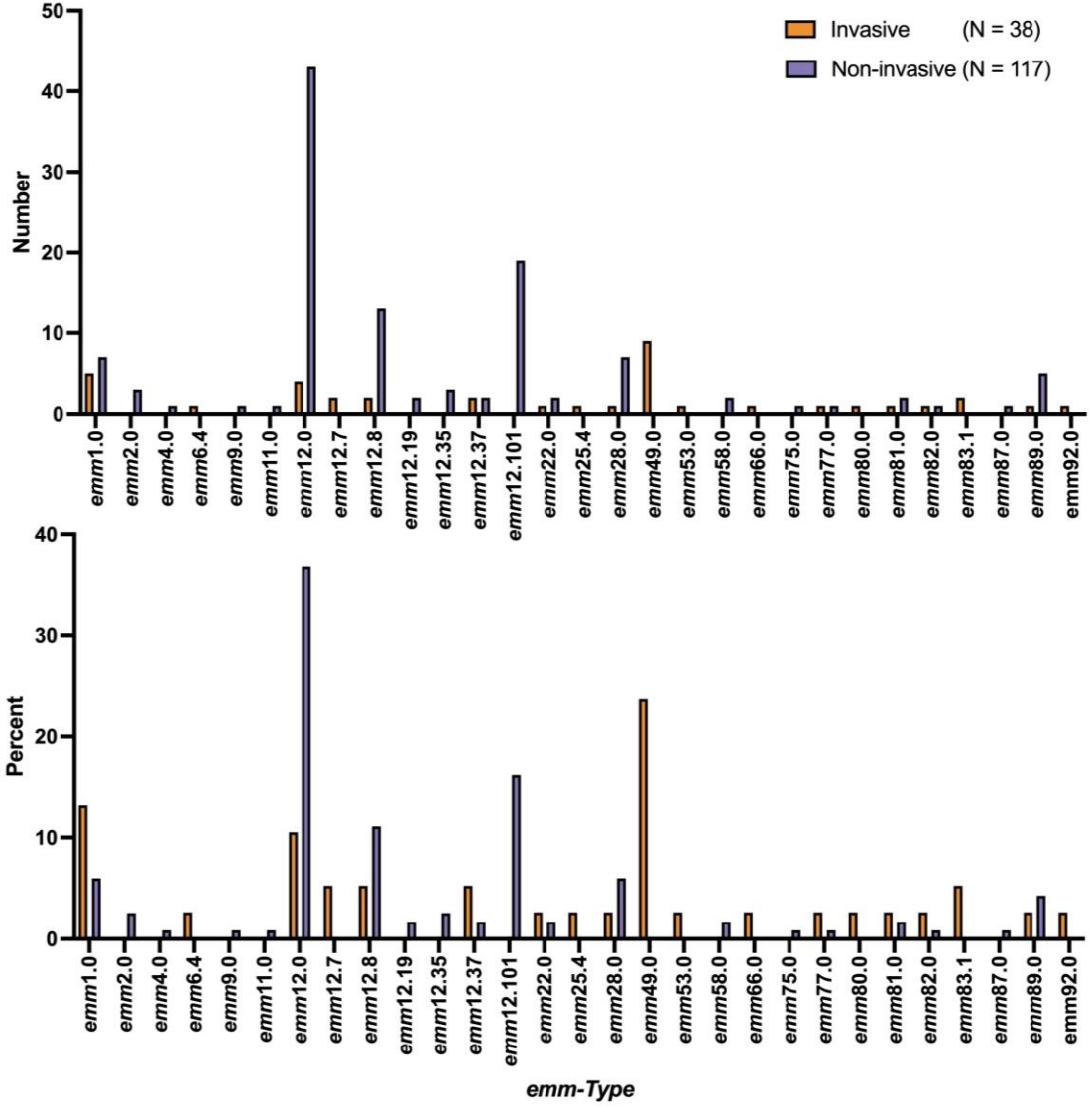


Key Gene Comparison



Phenotypic Assays

emm-(sub)type distribution in clinical isolates



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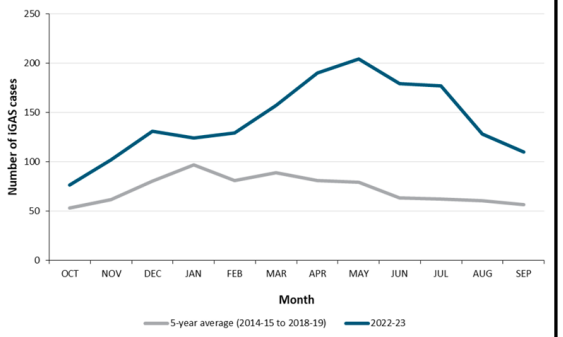


Data source: Ontario. Ministry of Health; 2024.[4]

Invasive

*emm12** = 26.36%

emm49 = 23.68%

emm1 = 13.16%

Non-Invasive

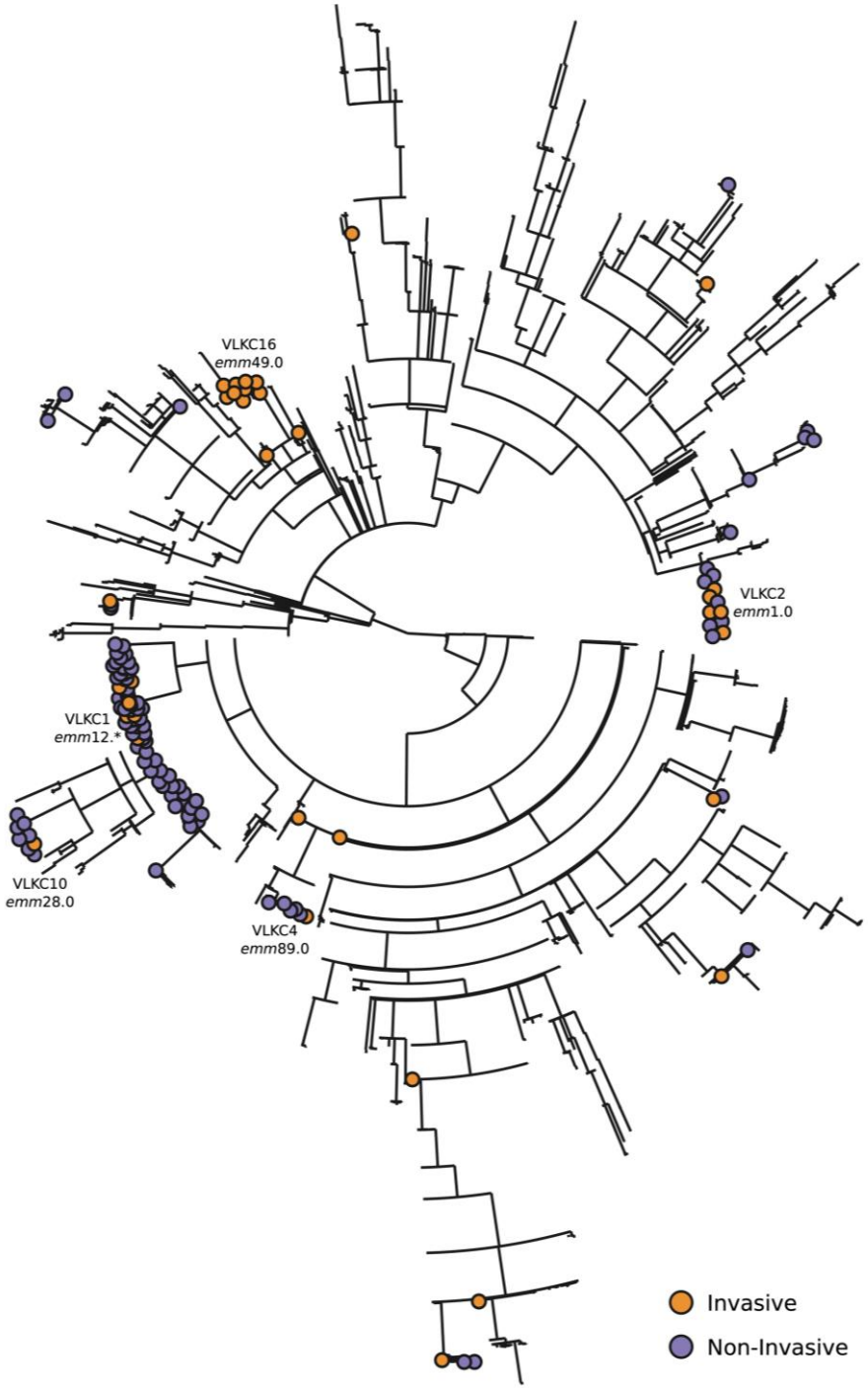
*emm12** = 70.09%

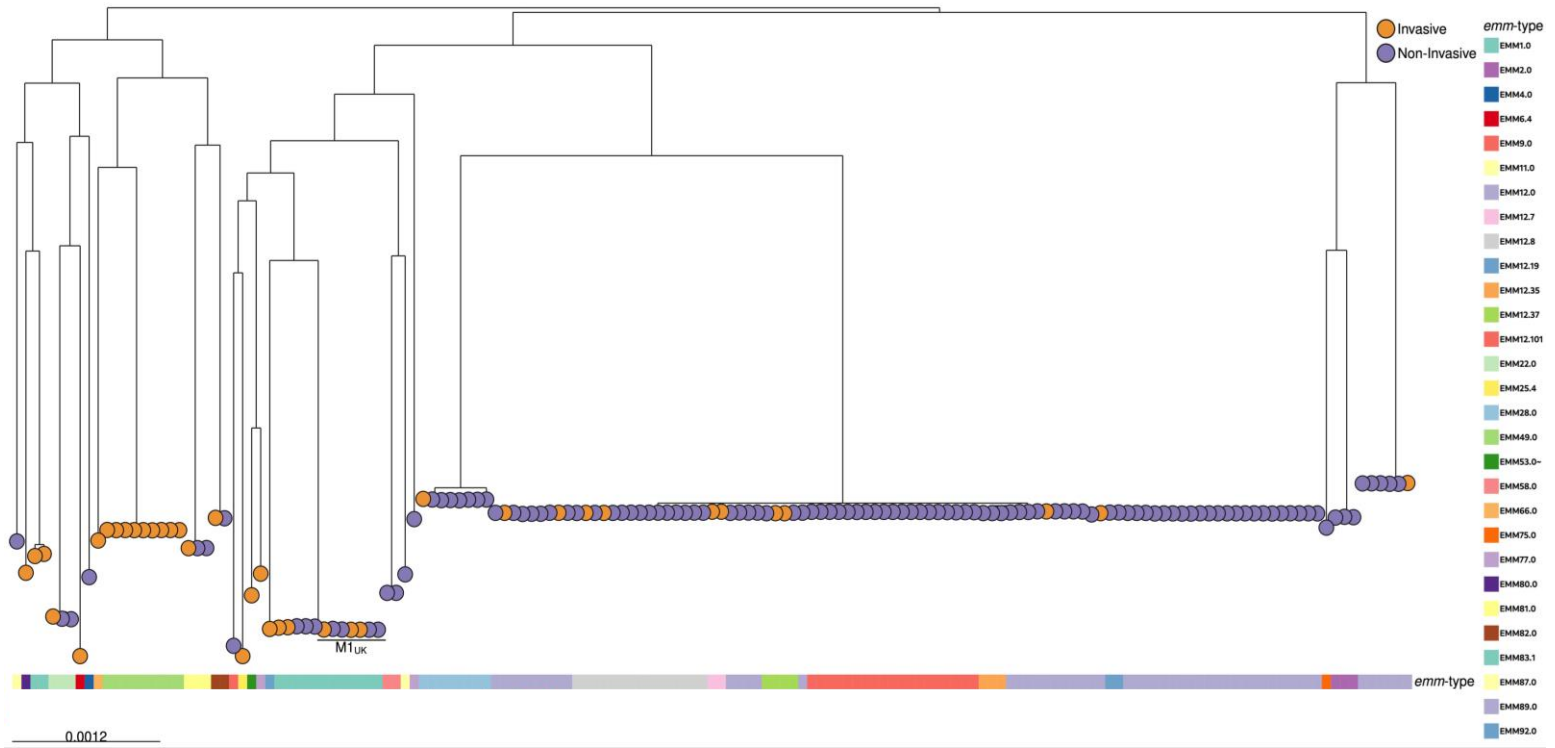
Table 6. Number (%*) of most commonly reported *emm* types among confirmed iGAS cases by age group: Ontario, 2022-23 season (October 1, 2022 – September 30, 2023) compared to the five pre-pandemic seasons (October 1, 2014 – September 30, 2019)**

Most commonly reported *emm* type by rank	2022-23 season: All cases	Previous five seasons: All cases	2022-23 season: cases age ≥ 18	Previous five seasons: cases age ≥ 18	2022-23 season: cases age < 18	Previous five seasons: cases age < 18
emm1	250 (19.5%)	480 (16.6%)	190 (16.7%)	395 (15.0%)	60 (42.0%)	85 (33.9%)
emm12	232 (18.1%)	172 (5.9%)	181 (15.9%)	155 (5.9%)	51 (35.7%)	17 (6.8%)
emm49	114 (8.9%)	82 (2.8%)	109 (9.6%)	77 (2.9%)	5 (3.5%)	5 (2.0%)
emm82	102 (8.0%)	34 (1.2%)	102 (9.0%)	28 (1.1%)	0 (0.0%)	6 (2.4%)
emm80	70 (5.5%)	19 (0.7%)	69 (6.1%)	19 (0.7%)	1 (0.7%)	0 (0.0%)
emm74	53 (4.1%)	237 (8.2%)	53 (4.7%)	231 (8.7%)	0 (0.0%)	5 (2.0%)
emm83	38 (3.0%)	35 (1.2%)	37 (3.2%)	35 (1.3%)	1 (0.7%)	0 (0.0%)
emm41	37 (2.9%)	20 (0.7%)	36 (3.2%)	20 (0.8%)	1 (0.7%)	0 (0.0%)
emm89	34 (2.7%)	164 (5.7%)	34 (3.0%)	157 (5.9%)	0 (0.0%)	7 (2.8%)
emm92	33 (2.6%)	9 (0.3%)	33 (2.9%)	9 (0.3%)	0 (0.0%)	0 (0.0%)
emm53	27 (2.1%)	142 (4.9%)	27 (2.4%)	142 (5.4%)	0 (0.0%)	0 (0.0%)
emm77	27 (2.1%)	59 (2.0%)	26 (2.3%)	59 (2.2%)	1 (0.7%)	0 (0.0%)
Other	266 (20.7%)	1,441 (49.8%)	242 (21.2%)	1,315 (49.8%)	23 (16.1%)	126 (50.2%)
Total with *emm* type	1,283 (75.2%)	2,894 (67.0%)	1,139 (75.0%)	2,642 (67.3%)	143 (76.9%)	251 (64.4%)
Total without *emm* type	424 (24.8%)	1,426 (33.0%)	380 (25.0%)	1,286 (32.7%)	43 (23.1%)	139 (35.6%)
Total	**1,707 (100%)**	**4,320 (100%)**	**1,519 (100%)**	**3,928 (100%)**	**186 (100%)**	**390 (100%)**

Data source: Case data: Ontario. Ministry of Health;2024.[4]
Note: *Emm* type percentages are among cases with *emm* type information available.
**Cases with an unknown age are excluded from the age-related columns in this table.

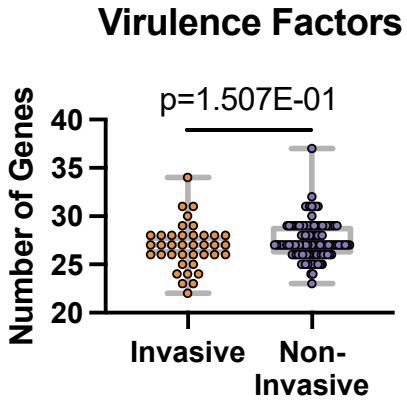
Minimum Spanning Tree and Core Genome Phylogeny

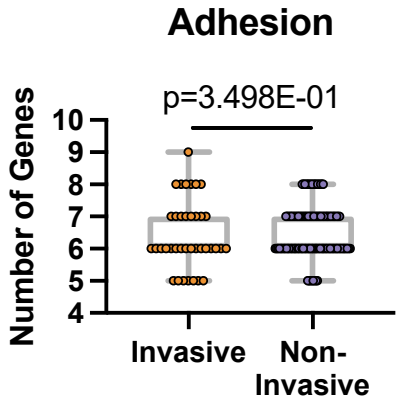


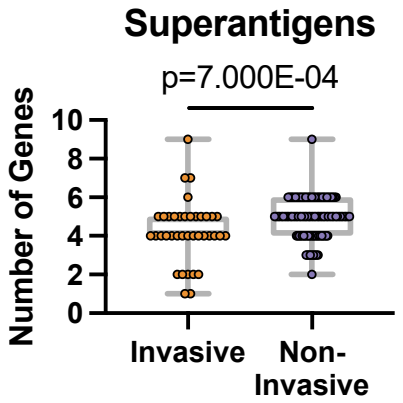


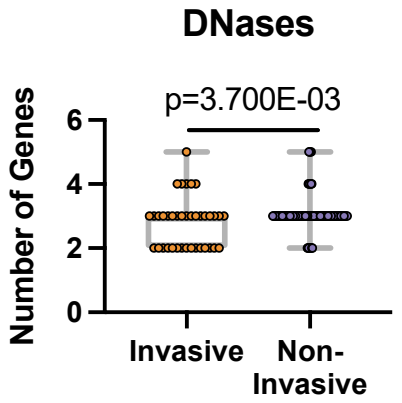
Non-invasive isolates have more SAg and DNase genes

Category	Subcategory	Gene
Virulence Factors	M & M-like proteins	emm
		enn
		mrp
	Capsule	hasA
		hasB
		hasC
	Superantigens	speA
		speC
		speG
		speH
		speI
		speJ
		speK
		speL
		speM
		speQ
		speR
		ssa
		smez
	DNases	spnA
		spdB/mf1
		sda1
		sda2
		spd1/mf2
		spd3/mf3
		spd4/mf4
		sdn
	Leukocidins & associated genes	sagA
		slo
		nga
	Hyaluronidases	hlyA
		hylP
	Other Proteases and Virulence Factors	endoS
		scpA
		scpC
		sodA
		cppA
		grab
		ideS/Mac
		sic
		speB
		s5na
		cfa
		htrA/degP
		ska
		slaA
		spyA
Adherence and other binding proteins	Fibronectin Binding Proteins	fbaA
		fbaB
		fbp54
		sfbI/prtF1
		sfbII/sof
		prtF2
		sfbx
	Collagen Binding Proteins	cpa
	Laminin Binding Proteins	lmb
	Plasmin Receptor	plr/gapA
	Collagen-like Proteins	sclA
		sclB







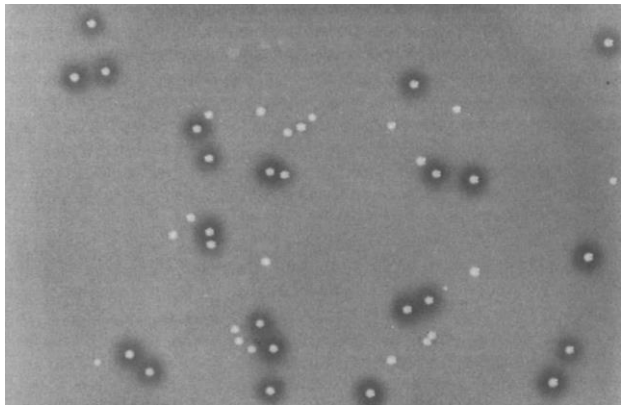


More prominent in INVASIVE isolates		
VF	M-like	*enn*
		mrp
	SAg	*speA*
	Other	*ideS/Mac*
Adhesion	FBP	*fbaA*
		fbaB

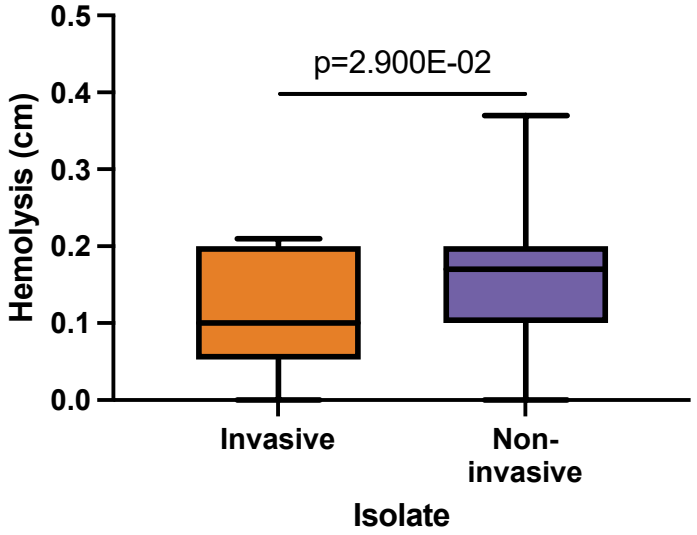
More prominent in NON-INVASIVE isolates		
VF	SAg	*speC*
		ssa
		smez
	DNase	*spd1*
	Other	*hylP*
		endoS
		grab
Adhesion	FBP	*sfbI*
		prtF2

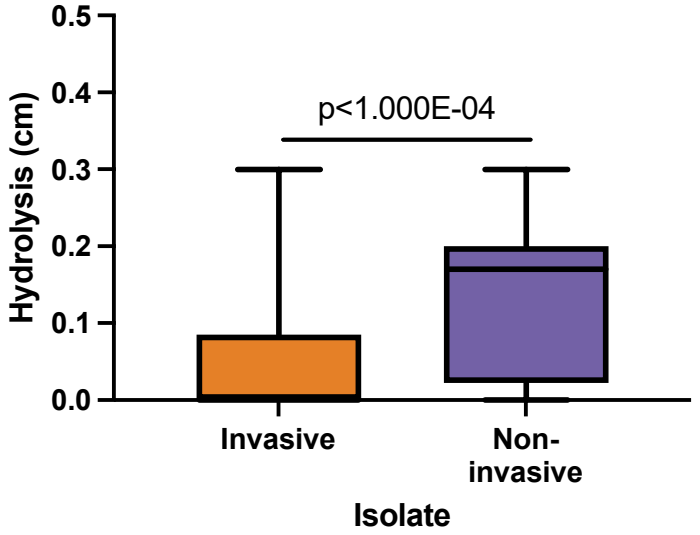
Non-invasive isolates produce more lytic and proteolytic factors

Blood Agar



Milk Agar





Mutations in key two-component system only found in invasive isolates

CovS

```
1     MENQKQKQKK YKNSLPKRLS NIFFVLFFCI FSAFTLIAYS STNYFLLKKE KQSVFQAVNI
61    VRVRLSEVDS NFTLENLAEV LYKNDKTHLR IDDRKGSRVI RSERDITNTL DANQDIYVYN
121   IDKQMIFTTD NEESSPGLHG PIGRVYHDHI EDQYRGFSMT QKVYSNRTGK FVGYVQVFHD
181   LGNYYVIRAR LLFWLLVVEL FGTSLAYLII LITTRRFLKP LHNLHEVMRN ISENPNNLNL
241   RSDISSGDEI EELSVIFDNM LDKLETHTKL QSRFISDVSH ELRTPVAIIK GHIGLLQRWG
301   KDDSDILEES LTATAHEADR MAIMINDMLD MVRVQGSFEG HQNDMTVLED SIETVVGNFR
361   VLREDFIFTW QSENPKTIAR IYKNHFEQAL MILIDNAVKY SRKEKKIAIN LSVTGKQEAI
421   VRVQDKGEGI SKEDIEHIFE RFYRTDKSRN RTSTQAGLGI GLSILKQIVD GYHLQMKVES
481   ELNEGSVFIL HIPLAQSKES
```

Functional Domain
TM1/2
HAMP
HisKA
HATPase

Isolate Identifier	Amino Acid(s) Deleted	Location
23SC_014M0062_S3_L001	1 - 46	TM1
23SG_034M0106_S8_L001	1 – 46	TM1
23SH_038M1879_S10_L001	1 – 46	TM1
23SH_071M0020_S18_L001	1 – 46	TM1
23SC_035M0015_S9_L001	137	Non-Functional Region
23SC_083M0072_S18_L001	405 - 412	HATPase
23SH_005M1638_S1_L001	405 - 412	HATPase

Invasive
7/38 = 18.4%

Non-Invasive
0/117 = 0%

Antimicrobial resistant genes in clinical isolates

Antibiotic class	Gene	Invasive (*N* = 38)		Non-invasive (*N* = 117)		*P* value
		Number	Percent	Number	Percent	
Aminoglycoside	*ANT (6)-Ia*	2	5.26%	0	0.00%	5.890E−02
	APH(3′)-IIIa	2	5.26%	3	2.56%	5.967E−01
Trimethoprim	dfrG	1	2.63%	0	0.00%	2.452E−01
Macrolide	mefA	2	5.26%	1	0.85%	1.490E−01
Macrolide/streptogramin	msrD	2	5.26%	1	0.85%	1.49E−01
Macrolide/lincosamine/streptogramin	ermA	1	2.63%	3	2.56%	1.000E0
	ermB	0	0.00%	5	4.27%	3.349E−01
	ermT	2	5.26%	0	0.00%	5.890E−02
Streptothricin	Sat4	2	5.26%	3	2.56%	5.967E−01
Tetracyclines	tetM	5	13.16%	7	5.98%	1.685E−01
	tetO	0	0.00%	1	0.85%	1.000E0

Conclusion

WGS: both invasive and non-invasive isolates represented across a diverse set of lineages

Invasive

*emm12** = 26.36%

emm49 = 23.68%

emm1 = 13.16%

Non-Invasive

*emm12** = 70.09%

Differing prevalence of SAg, DNases and single VF/Adh.

More prominent in NON-INVASIVE isolates		
VF	SAg	*speC*
		ssa
		smez
	DNase	*spd1*
	Other	*hylP*
		endoS
		grab
Adhesion	FBP	*sfbI*
		prtF2

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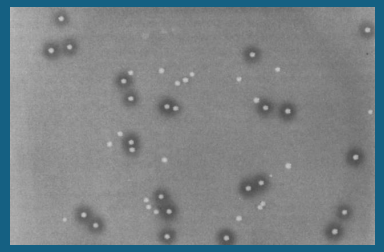
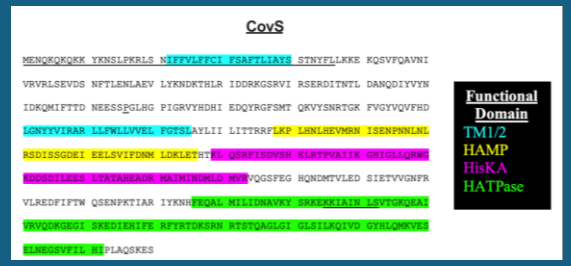


Non-Invasive isolates produced more lytic and proteolytic factors



Only Invasive isolates had mutations in *covS* gene

Future Directions

- Expand sample population to increase sample numbers and strengthen analyses
- Gather patient data to integrate host factors into overall findings
- Assess virulence factor/adhesin transcription/production using additional assays:
  - RNA sequencing
  - Assess protein production via Western blot/multiplex assay
  - *in vivo* animal models
- Phenotypic AST testing of isolates

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Kevin Katz
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SHL
SHARED HOSPITAL LABORATORY

Group A Streptococcus in Children: A comparison of invasive and non-invasive isolates

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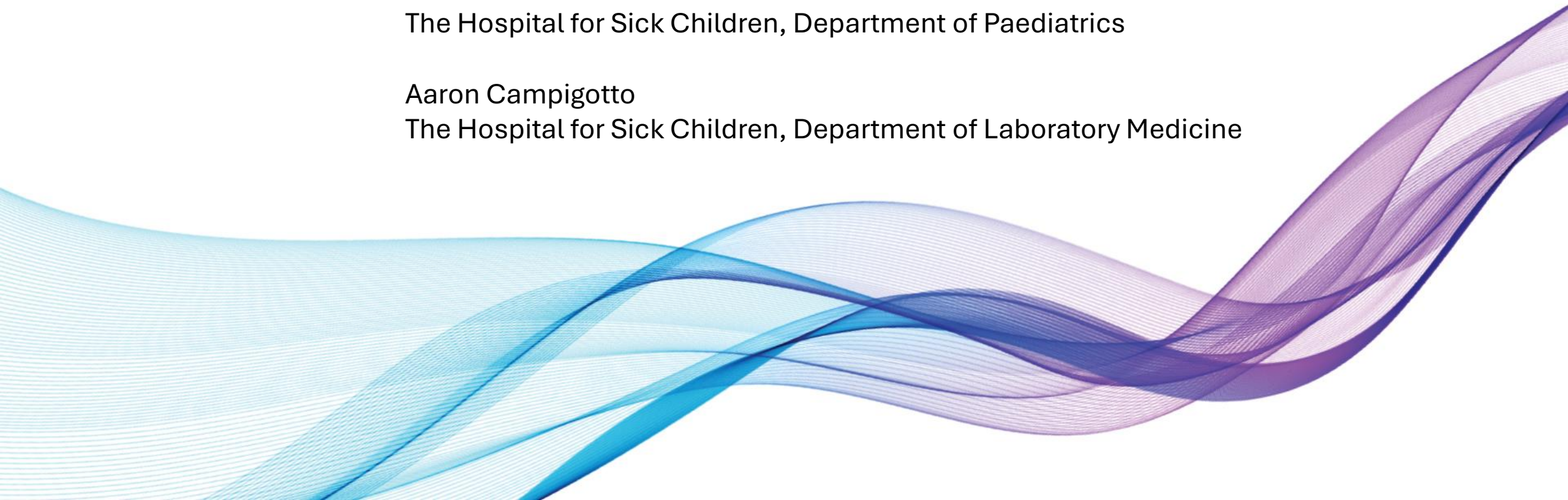
Aaron Campigotto
The Hospital for Sick Children, Department of Laboratory Medicine



Learning objectives

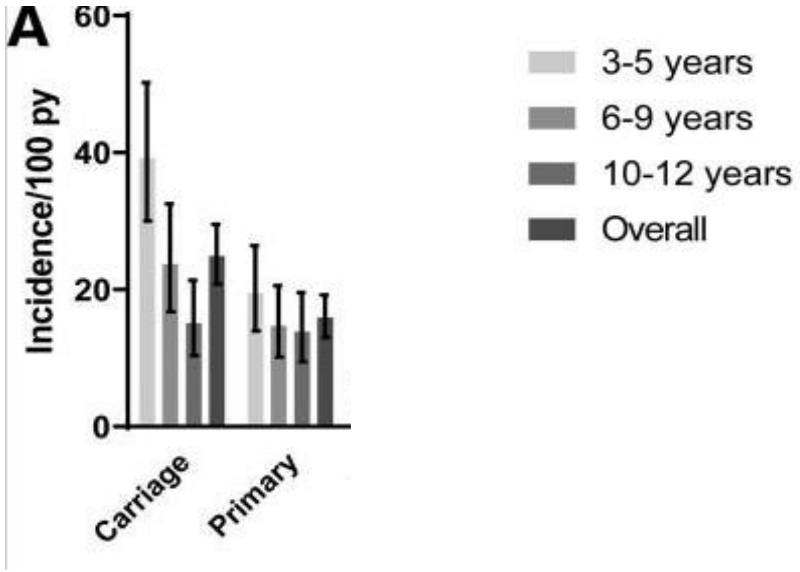
Describe epidemiology of invasive and non-invasive Group A Streptococcus (GAS) in children

1. Understand the clinical presentations of invasive and non-invasive GAS disease

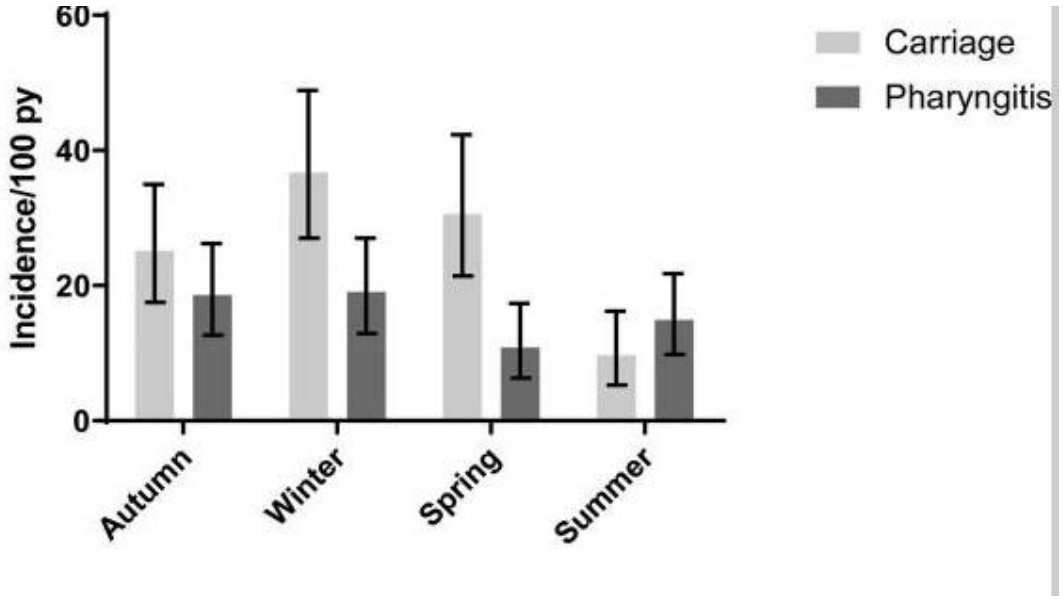
2. Understand circulating GAS *emm* types in this population and describe the *emm* types based on invasive and non-invasive clinical presentations

Colonization of GAS in children: Potential confounder?

- Colonization of GAS within the pharynx in up to 20% of children



- Seasonality present when assessing for colonization



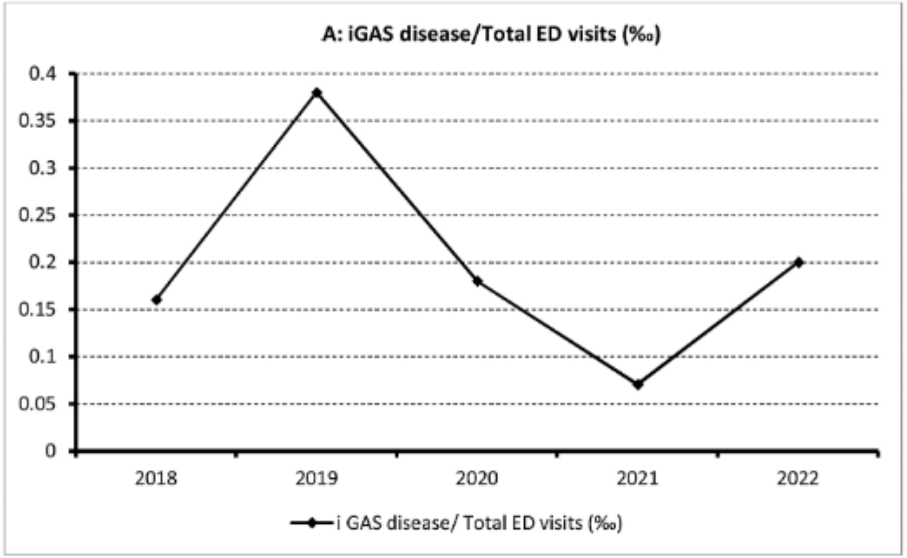
Frenck et al, PIDJ 2023

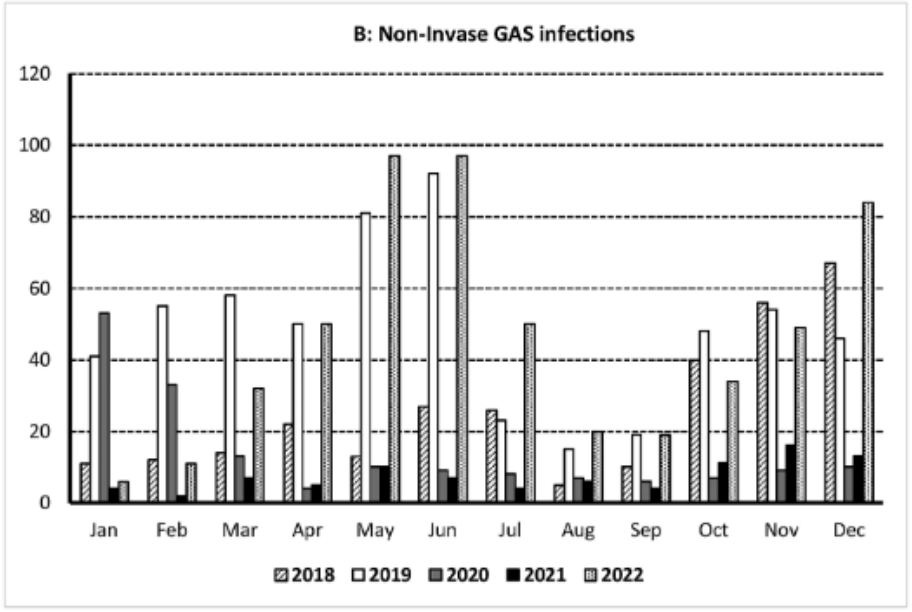
Prevalence of non-invasive GAS disease in children

Difficult to estimate given lack of reporting system and common clinical presentations including:

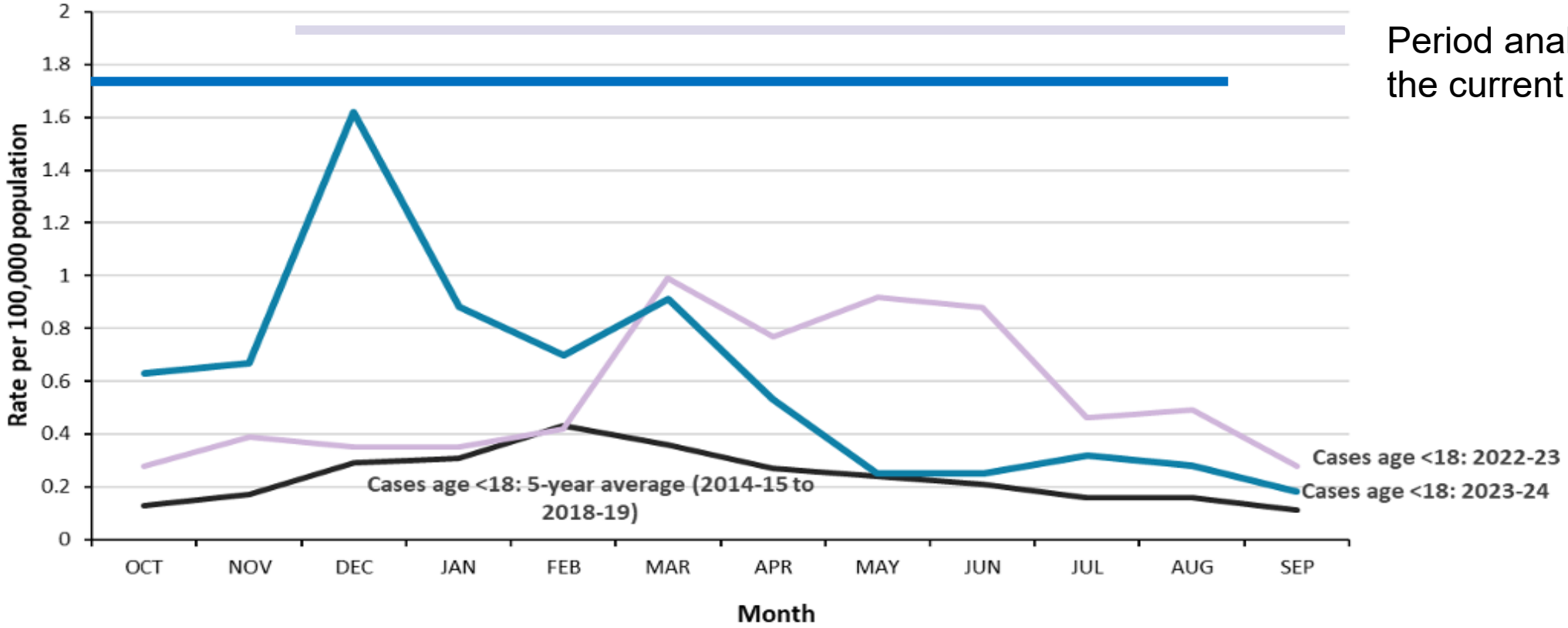
- Pharyngitis
- SSTI (e.g. impetigo)
- Scarlet fever

- Seasonality observed with non-invasive GAS disease





Rate of Invasive GAS reported in Ontario between 2022-2024 among children



Period analyzed within the current study

Clinical and bacterial characteristics of GAS

All clinical specimens with GAS isolated from SickKids between December 1, 2022 to August 31, 2024

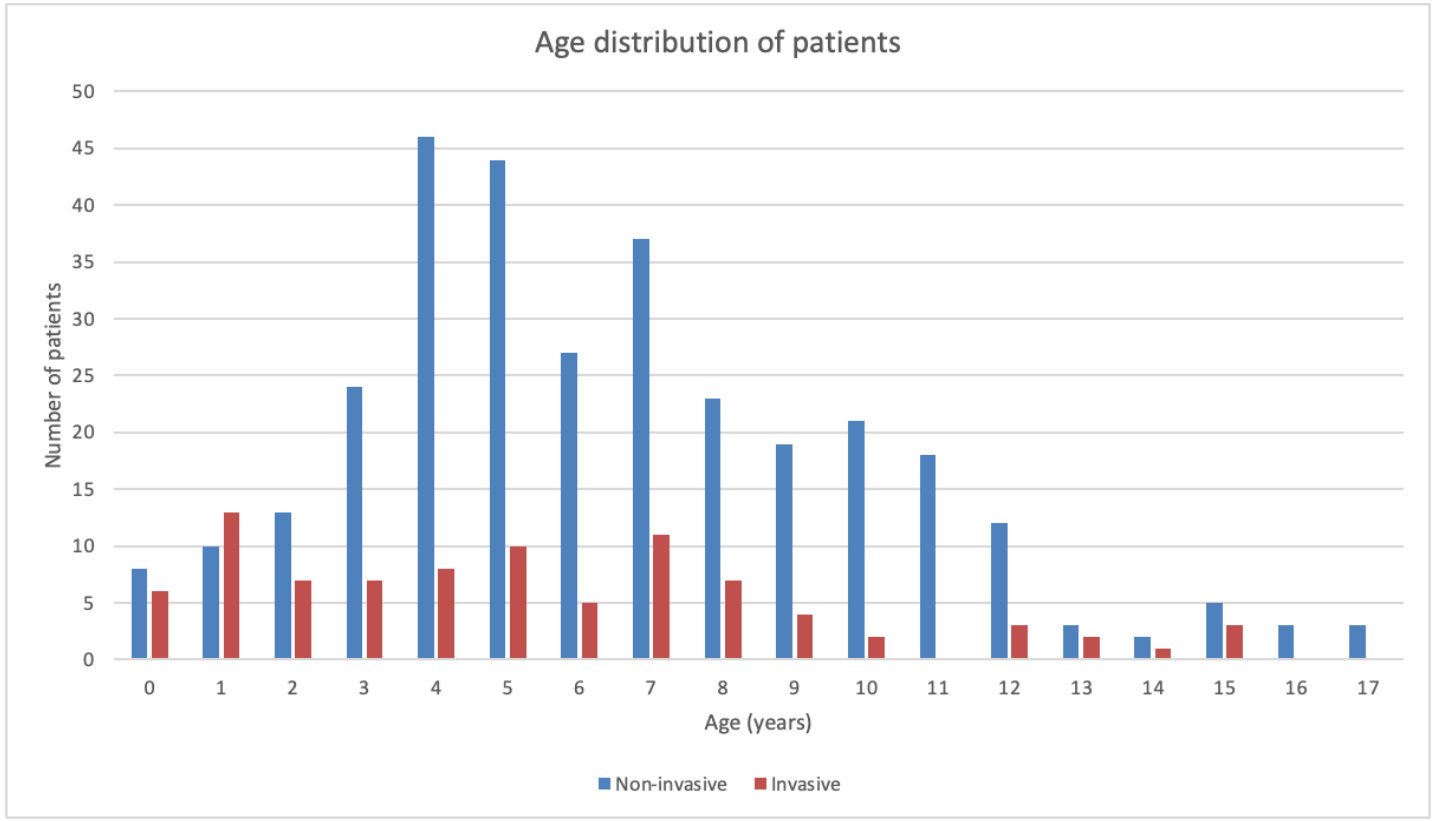
- Time period chosen to correspond with increase iGAS prevalence

Only 1 specimen per patient per 2-week period included in analysis

Patient and bacterial evaluation

- Clinical characteristics
  - Including age, collection site, clinical presentation
- Bacterial isolate whole genome sequencing (performed with ONT)
  - *emm*-type

Age distribution of patients with invasive and non-invasive GAS

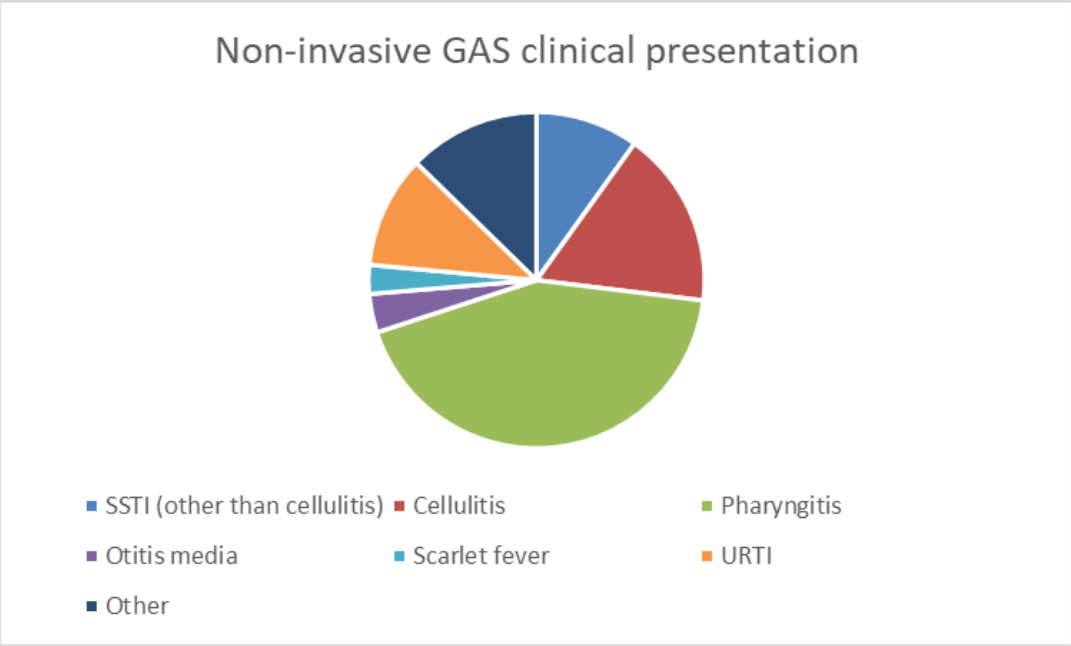


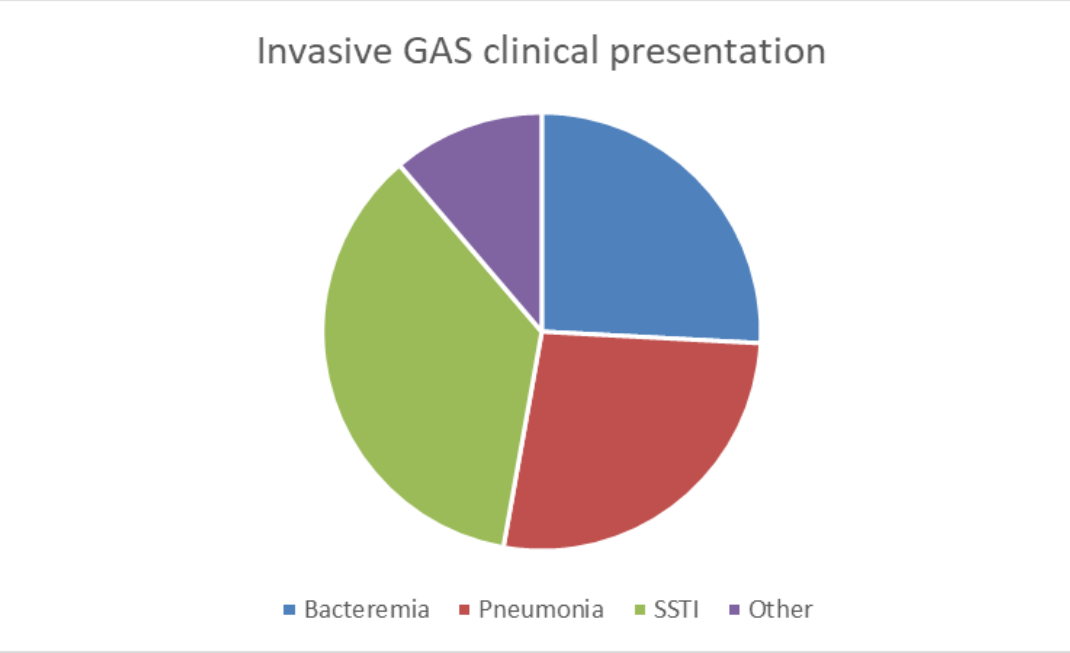
	Overall (n=408)	Non-invasive GAS (n=319)	Invasive GAS (n=89)
Age in years, median (IQR)	6 (4, 8.5)	6 (4, 9)	5 (2, 7)

Characteristics of patients with invasive and non-invasive GAS

	Overall (n=408)	Non-invasive GAS (n=319)	Invasive GAS (n=89)
Male sex, N (%)	226 (55%)	176 (55%)	50 (56%)
Underlying Medical Conditions, N (%)	147 (36%)	119 (37%)	28 (46%)
Eczema / Skin Condition	40 (10%)	36 (11%)	4 (4%)
Asthma / Resp	13 (3%)	12 (4%)	1 (1%)
Developmental	20 (5%)	12 (4%)	8 (9%)
Malignancy / Transplant	12 (3%)	10 (3%)	2 (2%)

Clinical presentation of GAS

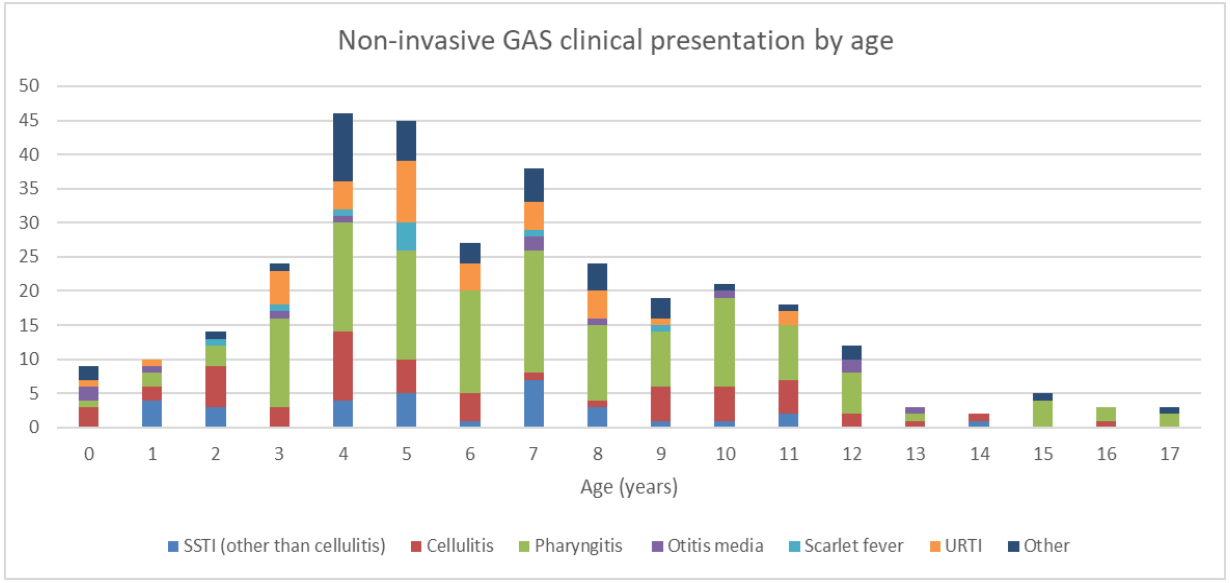


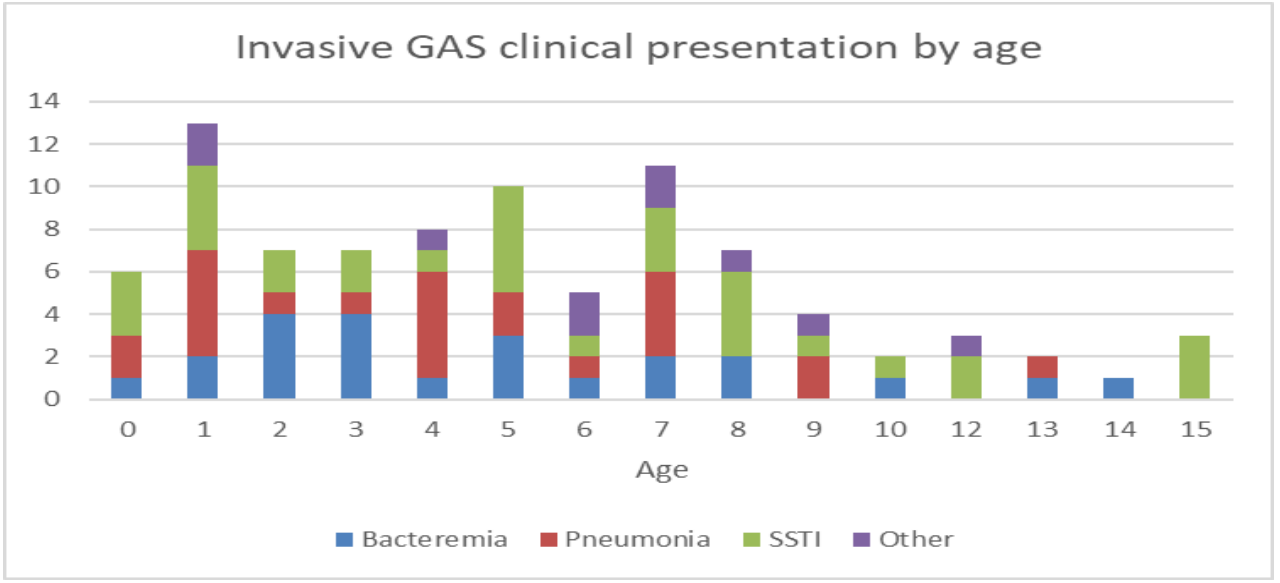


Total non-invasive isolates	88
WGS completed	81

Total invasive isolates	324
WGS completed	291

Clinical presentation of GAS (2)





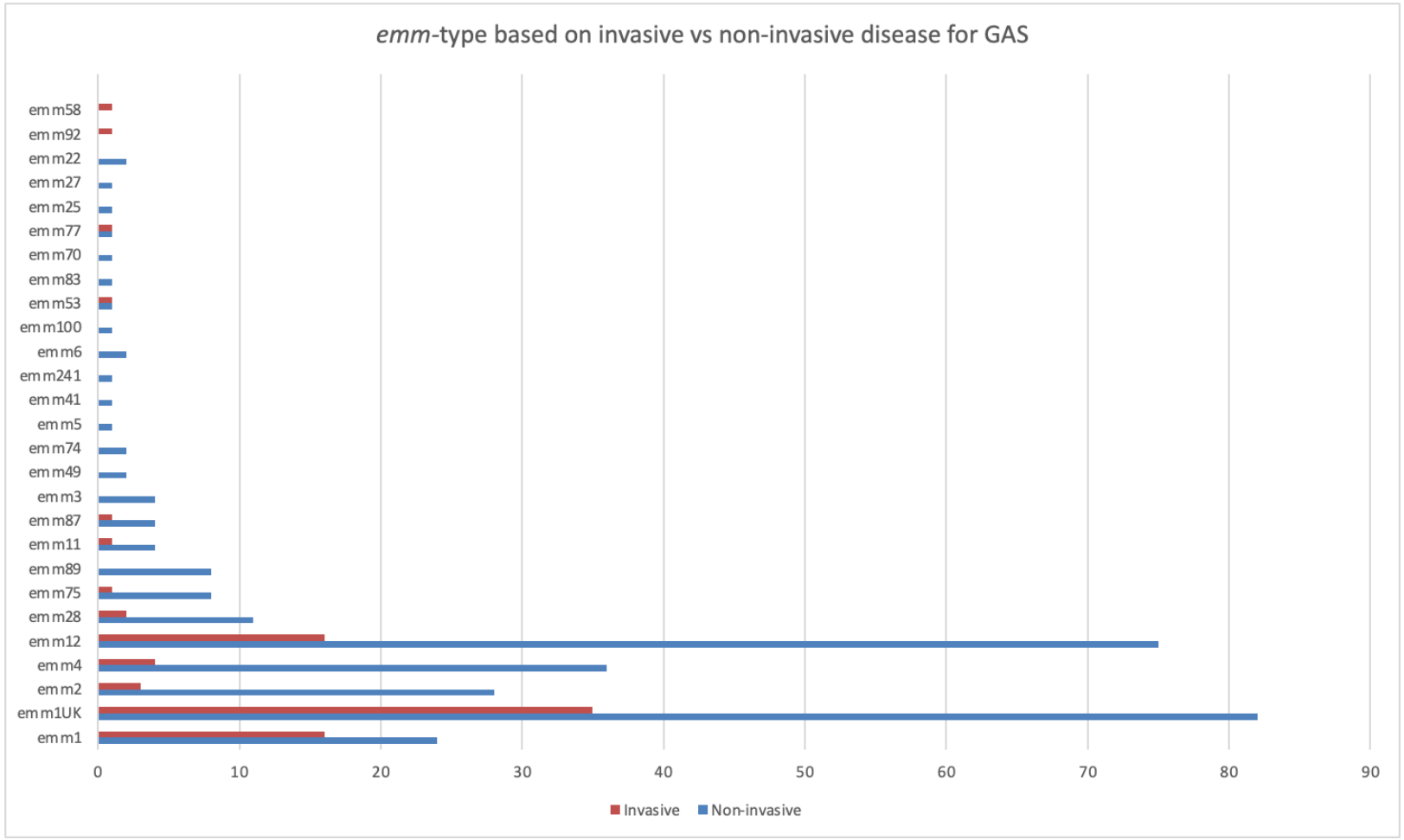
emm-type based on clinical presentation (invasive vs non-invasive)

Top 3 invasive *emm*-types:

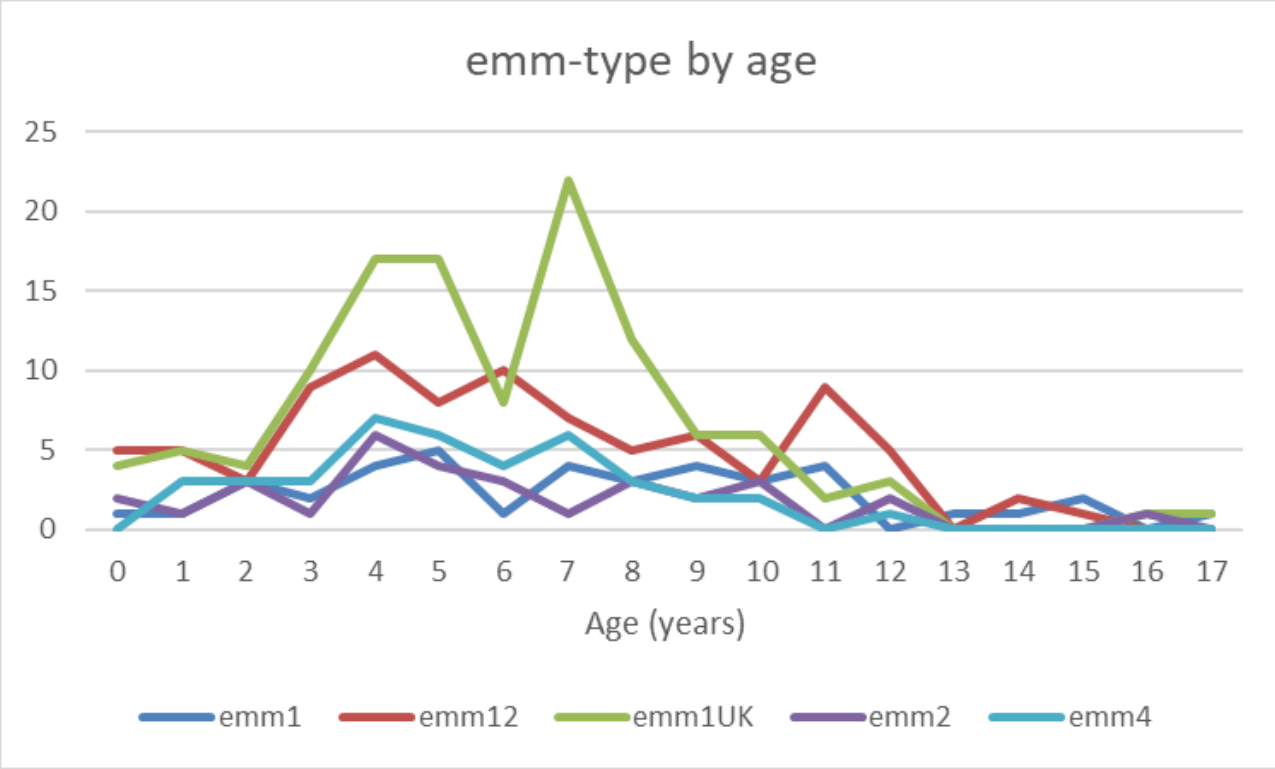
1. emm1UK
2. emm12
3. emm1

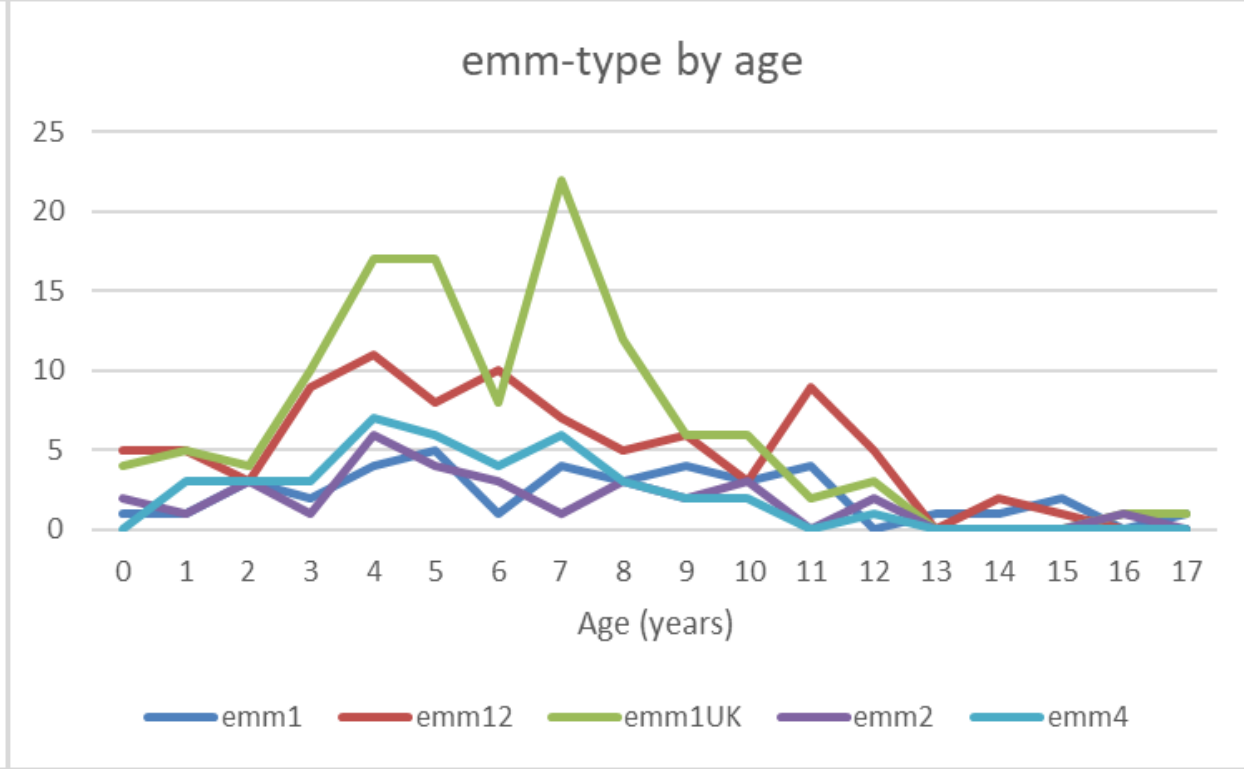
Top 3 non-invasive *emm*-types:

1. emm1UK
2. emm12
3. **emm2** (**emm4**, emm1)



Most common *emm*-type distribution by age

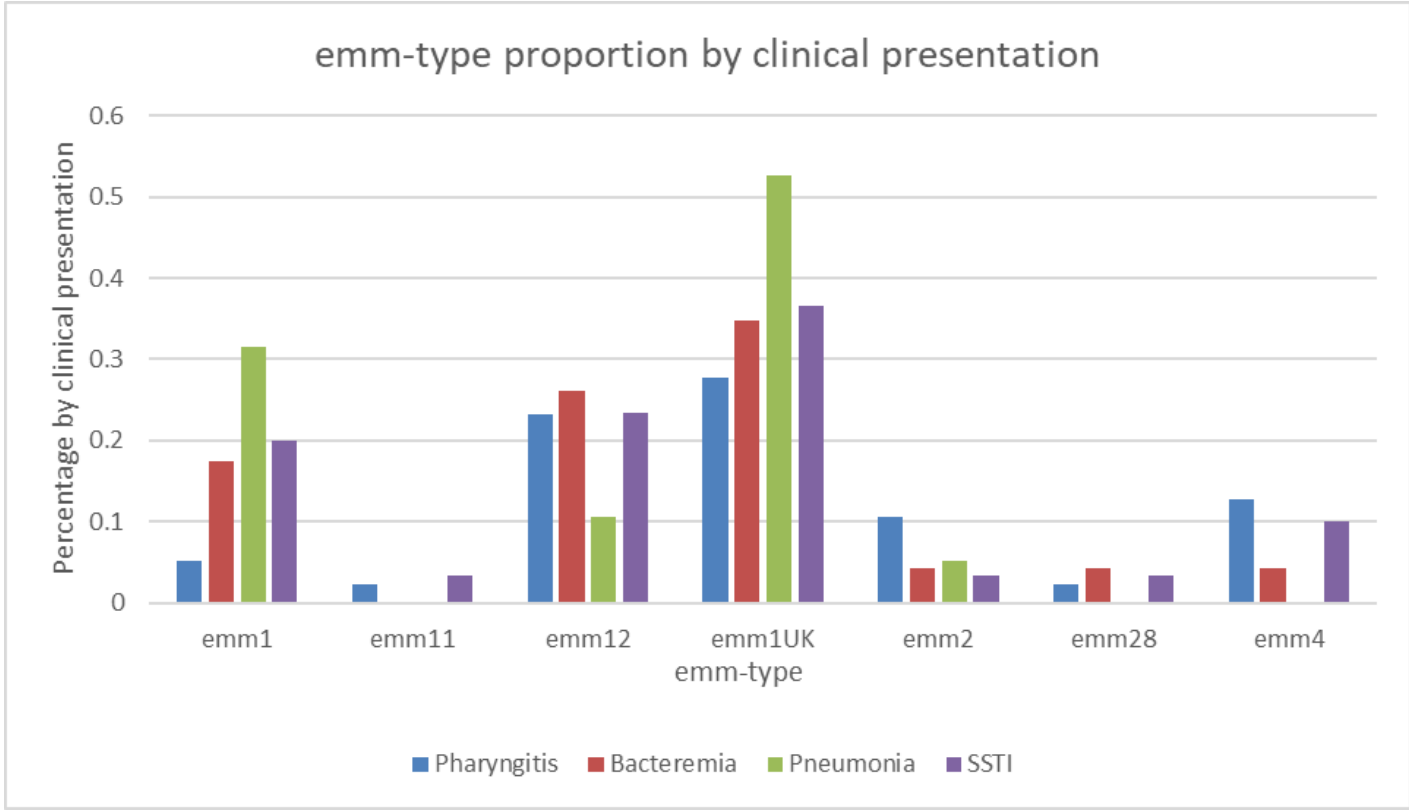




Proportion of *emm*-type by clinical presentation

Similar proportions observed with few exceptions:

- Decreased *emm*1 among pharyngitis
- Decreased *emm*12 among patients presenting with pneumonia
- Increased *emm*1UK among patients presenting with pneumoniae



Conclusion

*emm*1UK and *emm*12 were present in a similar proportion for invasive and non-invasive isolates

emm1UK predominate lineage in both clinical cohorts

*emm*1 was present in invasive isolates more than non-invasive isolates

e.g. Few emm1 isolates among children with pharyngitis

*emm*2 and *emm*4 was present in higher amounts among non-invasive isolates

e.g. Few *emm*2/*emm*4 invasive isolates were observed

TIBDN and iGAS Surveillance

Allison McGeer
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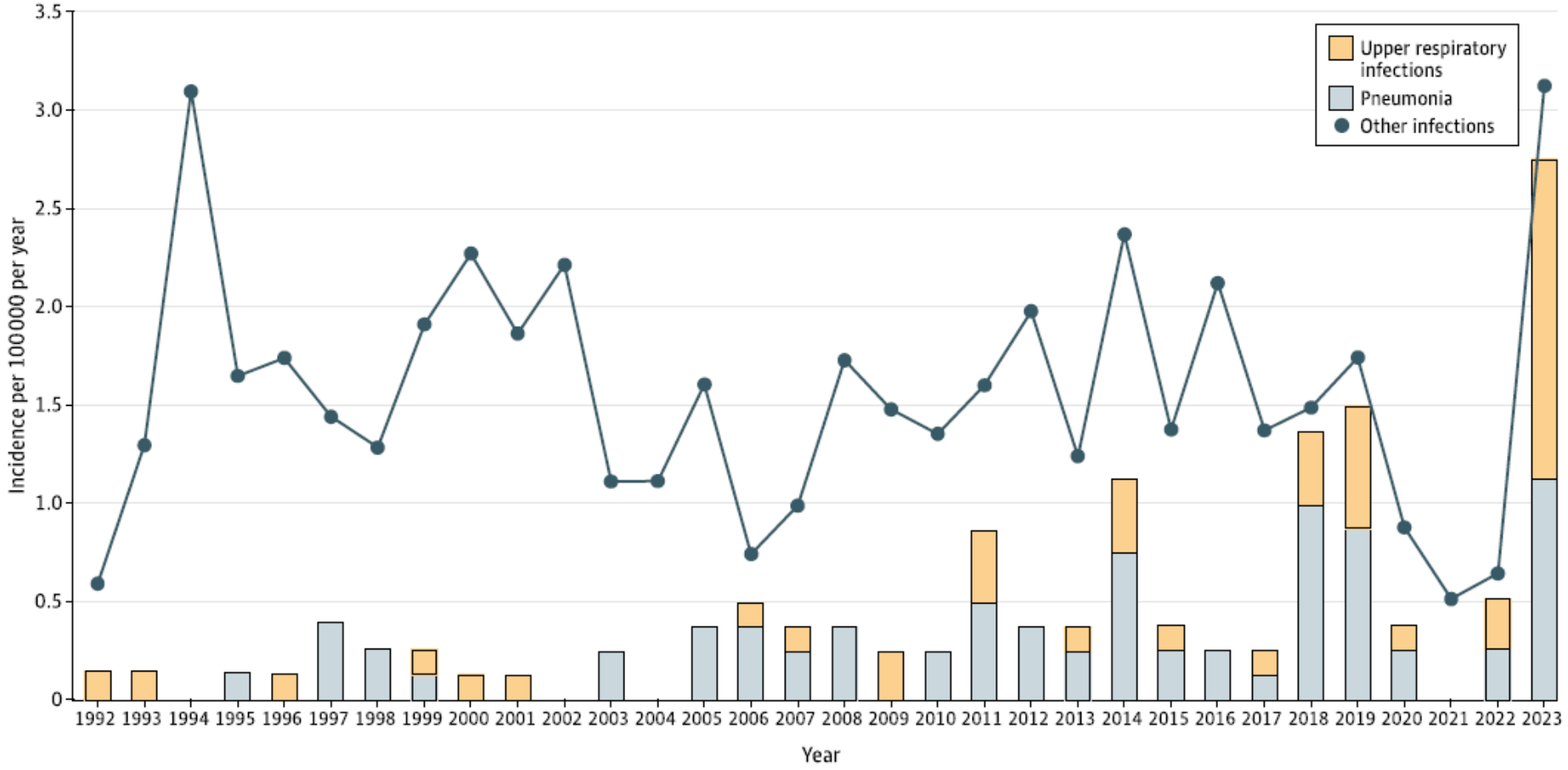
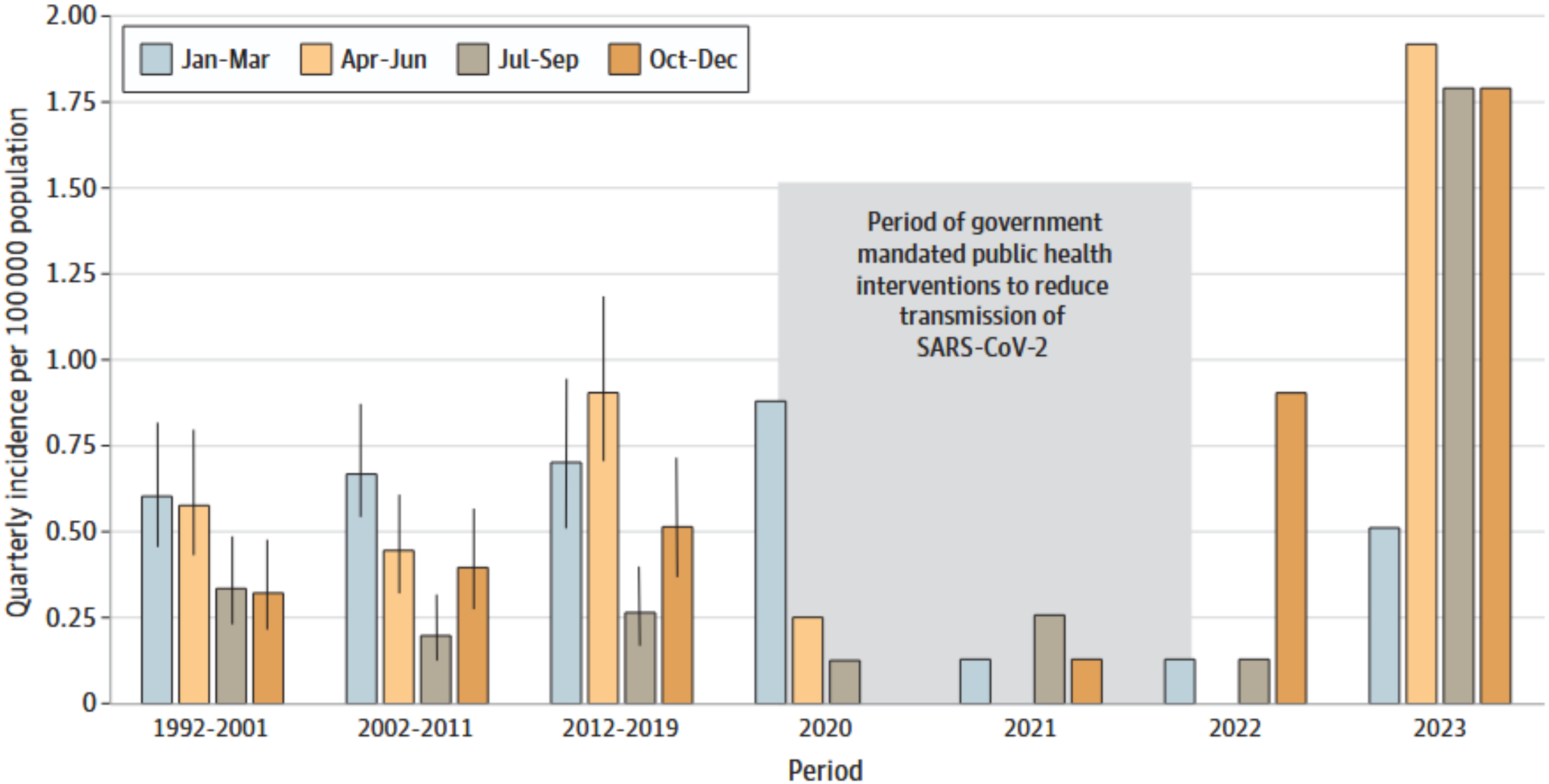
Dalla Lana School of Public Health
University of Toronto
Senior Clinician Scientist,
Lunenfeld Tanenbaum Research Institute,
Sinai Health System

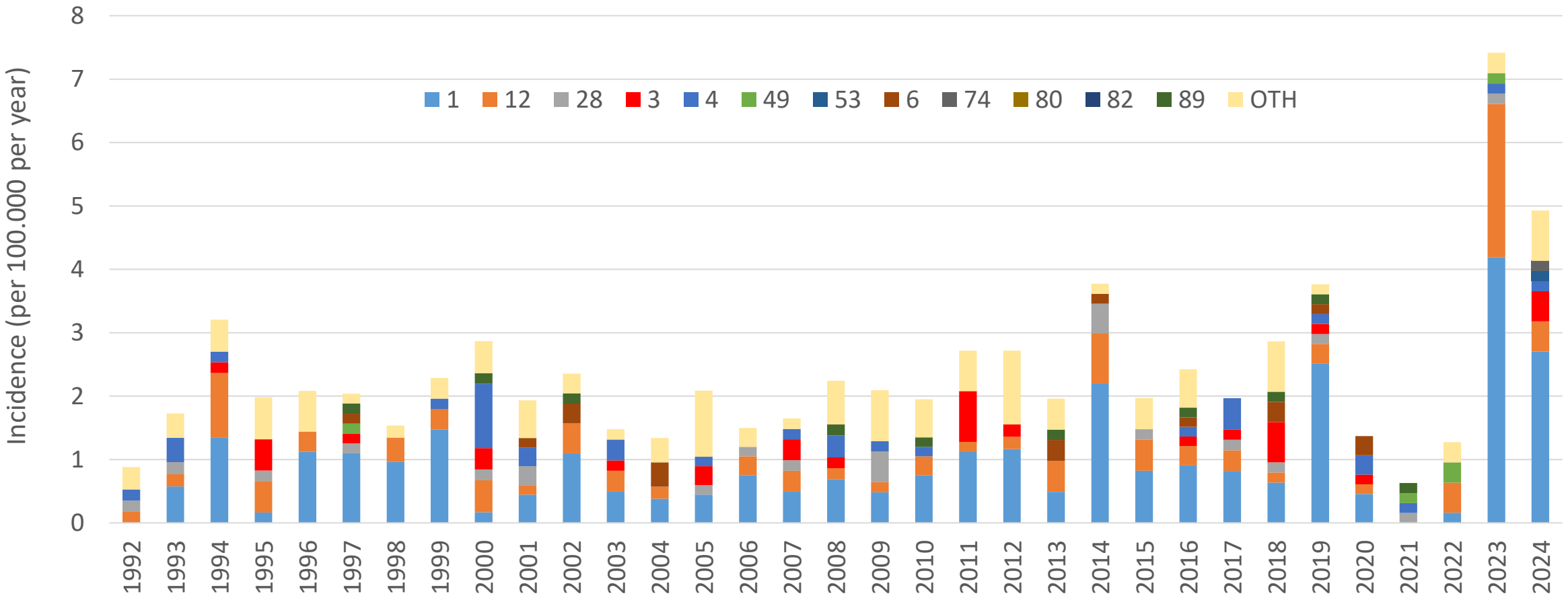
Incidence of pediatric invasive GAS disease, Toronto/Peel, 1992-2023



Seasonality of pediatric iGAS disease, Toronto/Peel, 1992-2023

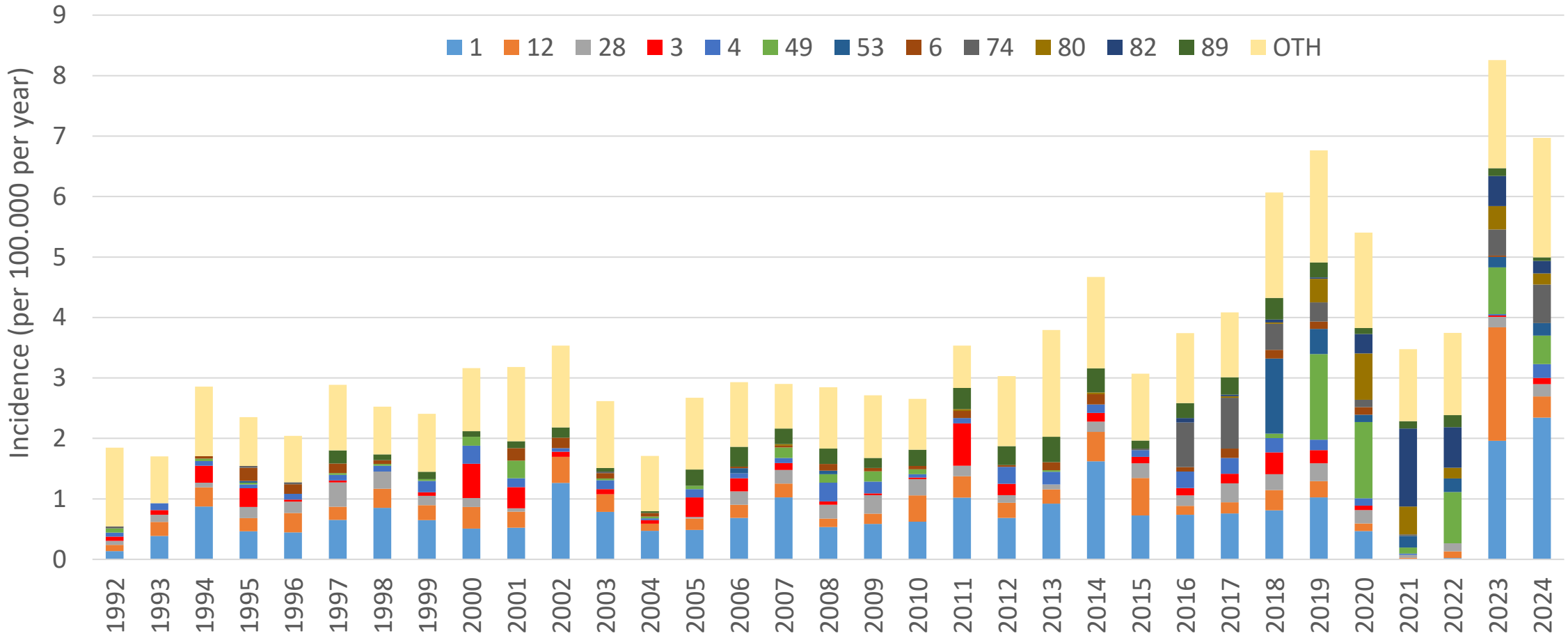


Incidence of iGAS, Toronto/Peel, Children (<15 years), 1992-2024

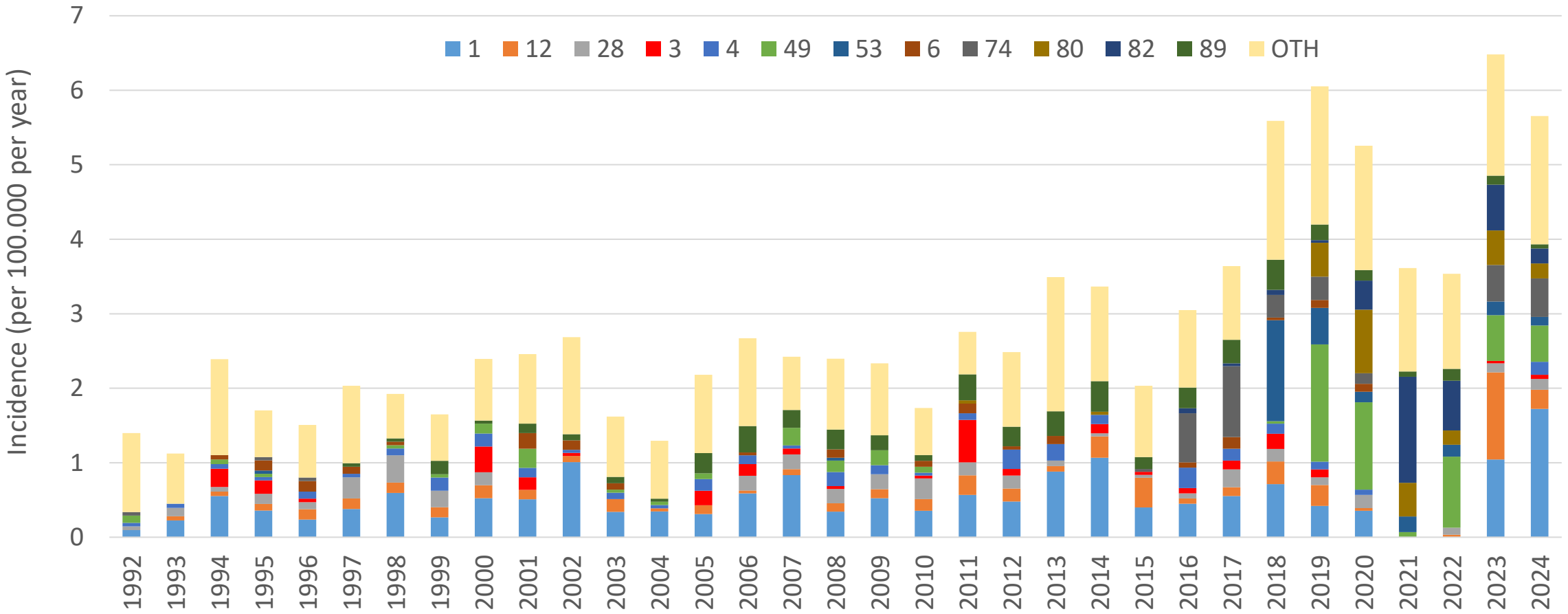


92.8% of emm types are included in the 30-valent GAS vaccine

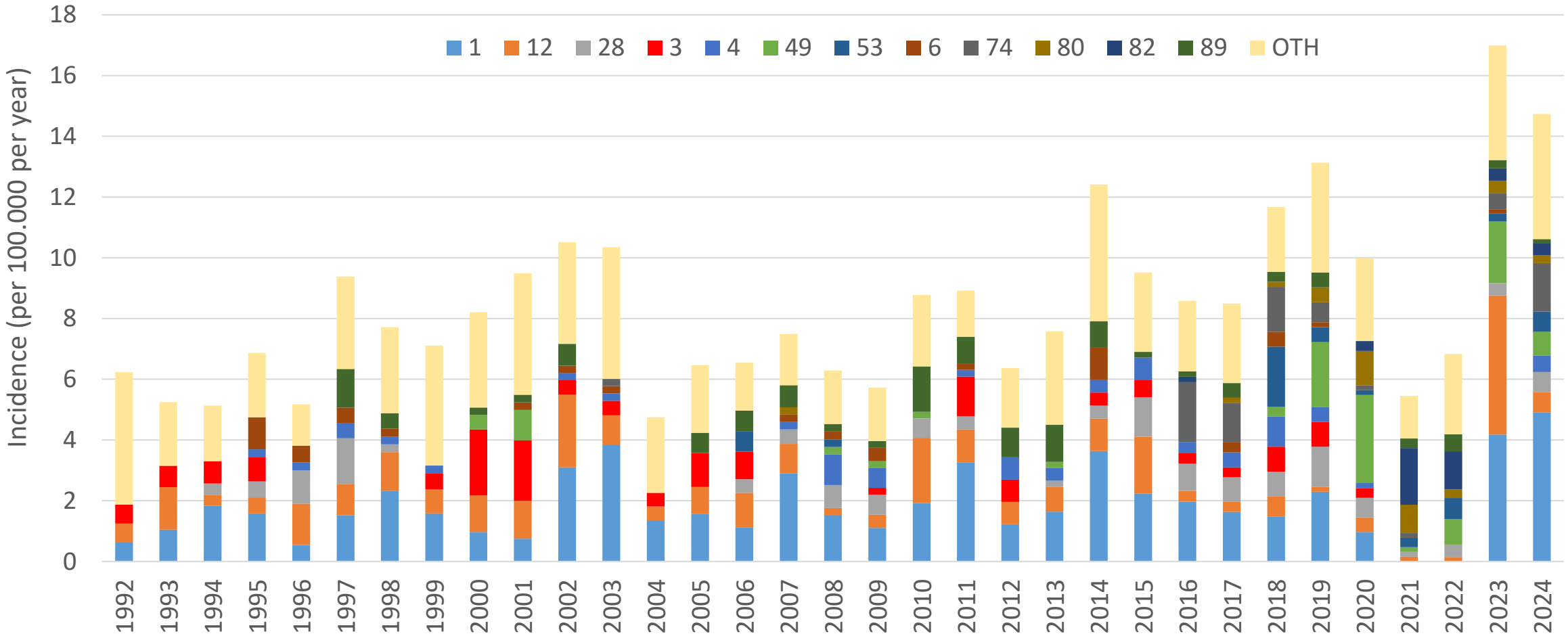
Incidence of iGAS, Toronto/Peel, All ages, 1992-2024



Incidence of iGAS, Toronto/Peel, Adults aged 15-64 years, 1992-2024



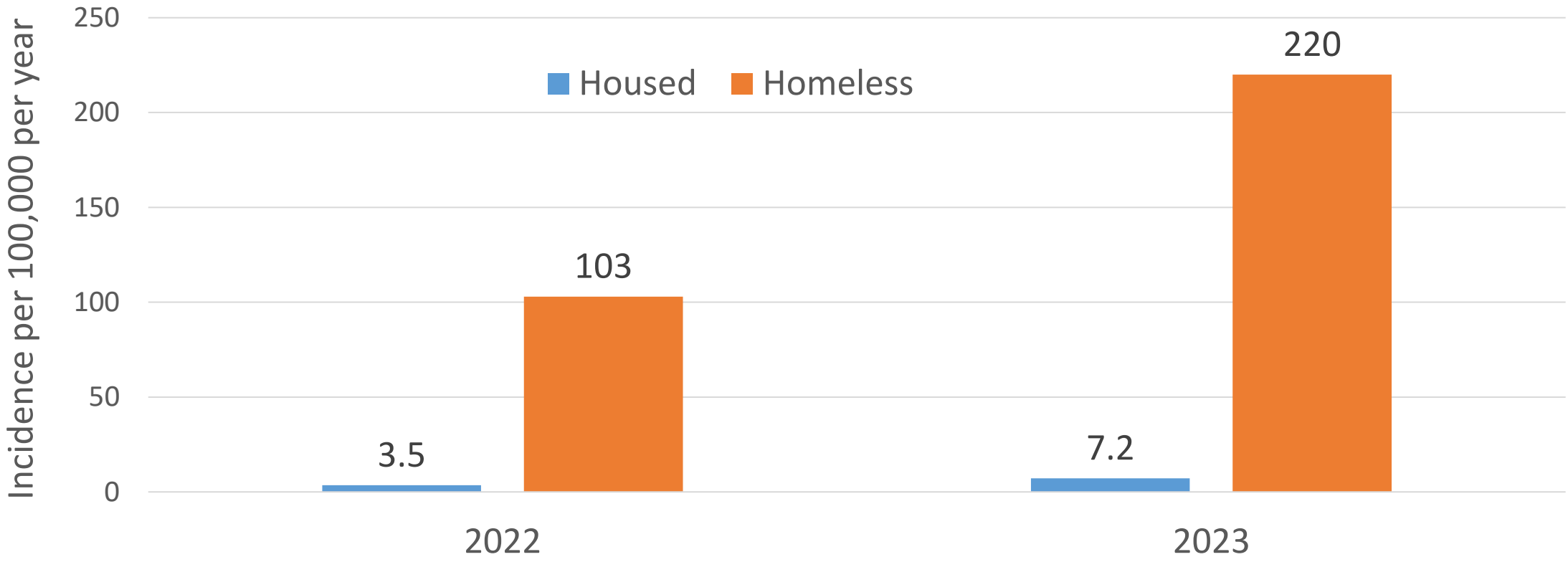
Incidence of iGAS, Toronto/Peel, Older adults (≥65 years), 1992-2024



What do you think will happen to iGAS in the next decade?

1. The current post-pandemic iGAS increase will settle, and the incidence will return to pre-pandemic levels
2. iGAS incidence will stabilize at or near 2024 levels
3. iGAS incidence will continue to increase
4. We will have a vaccine in less than 10 years, and iGAS will decline when a vaccine program is introduced.

Incidence of iGAS, housed and houseless adults, Toronto/Peel, 2022-2023



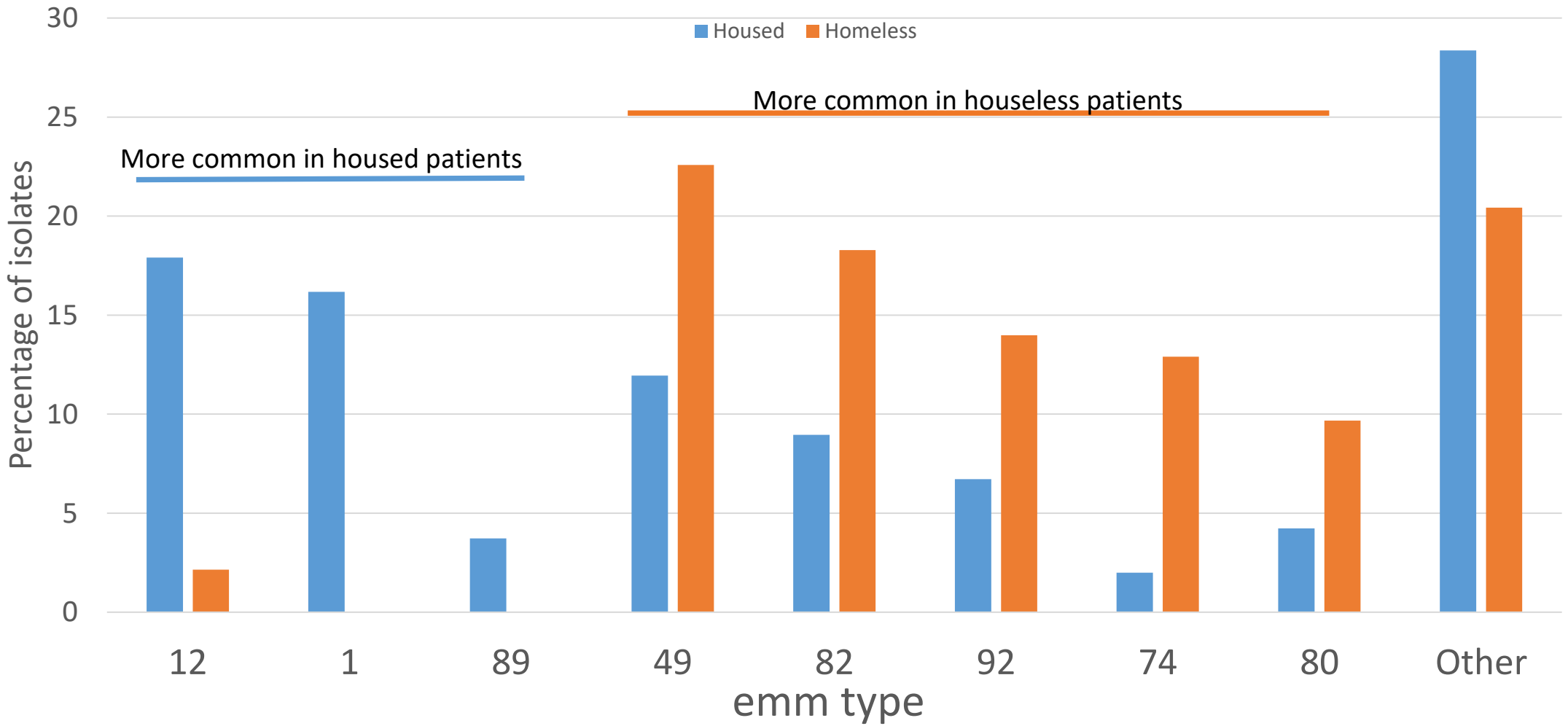
Characteristics of iGAS, housed and houseless adults, Toronto/Peel, 2022-2023

	Housed (n=408)	Homeless (n=94)	Odds Ratio$^{\alpha}$ (95%CI)	P-value
Age in years; median (IQR)	58 y (42-73)	47 (37-60)	-	0.008
Sex (n,% male)	256 (62.7)	68 (72.3)	-	0.10
Underlying Medical Conditions	N (%)	N (%)		
Diabetes mellitus	93 (22.8)	16 (17.2)		0.45
Pulmonary	61 (15.0)	11 (11.8)		0.81
Cardiac	94 (23.0)	8 (8.6)		0.10
Kidney	60 (14.7)	6 (6.4)		0.18
Autoimmune	28 (6.9)	0 (0.0)	NE	0.008
Immunocompromise	83 (20.3)	5 (5.3)	0.28 (0.11-0.72)	0.008
Substance Use				
Alcoholism	57 (14.0)	22 (23.7)		0.09
Current Smoker	88 (21.6)	50 (53.8)	3.49 (2.15-5.67)	<0.001
Intravenous Drug Use	30 (8.1)	33 (35.9)	5.15 (2.84-9.32)	<0.001
Infection Source and Risk Factors				
Acute Respiratory Illness in the Last 2 Weeks	25 (6.1)	2 (2.2)		0.17
Infection related to Healthcare or Delivery	23 (5.6)	3 (3.2)		0.46
Case related to another iGAS case	6 (1.7)	1 (1.6)		0.99
Recent Soft Tissue Trauma	84 (22.0)	20 (25.3)		0.76
Non Intact Skin	72 (17.6)	40 (43.0)	4.39 (2.60-7.40)	<0.001

Characteristics of iGAS, housed and houseless adults, Toronto/Peel, 2022-2023

	Housed (n=408)	Homeless (n=94)	Odds Ratio$^{\alpha}$ (95%CI)	P-value
Primary Clinical Diagnosis				
Soft Tissue Infection	192 (47.1)	58 (61.7)	1.81 (1.13-2.90)	0.01
Bacteremia without Focus	67 (16.4)	5 (5.3)	0.34 (0.13-0.87)	0.02
Upper Respiratory Tract Infection	44 (10.8)	3 (3.2)	0.25 (0.08-0.84)	0.023
Pneumonia	42 (10.3)	5 (5.3)		0.19
Arthritis or Bursitis	31 (7.6)	7 (7.4)		0.70
Osteomyelitis	9 (2.2)	9 (9.6)	4.65 (1.73-12.5)	0.002
Endocarditis	1 (0.2)	5 (5.3)	23.4 (2.56-213)	0.005
Other	19 (4.7)	1 (1.1)		0.16
Severity of Presentation				
Streptococcal Toxic Shock Syndrome	67 (16.4)	5 (5.3)	0.33 (0.13-0.85)	0.022
Necrotizing Fasciitis	36 (8.8)	6 (6.4)		0.48
Treatment/Outcome				
Hospitalized	370 (90.7)	81 (86.2)		0.59
Admitted to ICU	112 (27.5)	16 (17.0)		0.07
Died	69 (16.9)	4 (4.3)	0.31 (0.11-0.88)	0.03

Emm type distribution in iGAS
Housed vs. houseless patients, Toronto/Peel, 2022-2023



In Sum

- Most of the pandemic associated decrease in iGAS was associated with reduced transmission of *emm*1, which is more common in children than in adults
- The incidence of iGAS appears to be increasing
- Homeless adults are more than 30x more likely to develop iGAS compared to housed adults
- The most advanced GAS vaccine in development covers >90% of strains causing iGAS in children, and about 75% of all strains

Thank
You
Merci
Gracias
Danke
Spacibo
Hvala
Arigatô
KamSahHamnida
TerimaKasih
Takk
aDank
BarakAllahuFiik
Mèsi
Xièxie
Grazie
Chokrane
Kiitos
Dziakuju
ThintKo
Buznyg
TapadhLeat
Köszönöm
Murakoze
WaadMahadsantahay
Blagodaram
Rahmat
Matondo
Mercé
Taiku
Tanemirt
Tenki
Dakujem
Gràcies
Faleminderit
Efharisto
NajisTuke
Grandmercé
Nirringrazzjak
Bayarlalaa
Obrigado
Salamat
Saha
Multumesc
CámOn
Gracie
GratiasAgo
GratiasAgimus
KopKhunKha
Motashakkeram
Tak
Sagolun
Dankie
TänanVäga
Ačiū
Chnorakaloutioun
Barkal
Mauruuru
Grazzi
Gratzias
Aabhar
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Shukriya
Mamnun
aNiKíe
Toda
Dhanyavadagalu
Blagodaria
Akun
Mahalo
Graciñas
DankJuWel
Marahaba
Miigwetch
Spas
Wado
Asante
Misaotra
Akpe
Tanmirt
MaloTeOfa
Gràçie